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Structure-Based Design of Parasitic Protease Inhibitors

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Abstract—To streamline the preclinical phase of pharmaceutical development, we have explored the utility of structural data on the molecular target and synergy between computational and medicinal chemistry. We have concentrated on parasitic infectious diseases with a particular emphasis on the development of specific noncovalent inhibitors of proteases that play a key role in the parasites' life cycles. Frequently, the structure of the enzyme target of pharmaceutical interest is not available. In this setting we have modeled the structure of the relevant enzyme by virtue of its sequence similarity with proteins of known structure. For example, we have constructed a homology-based model of falcipain, the trophozoite cysteine protease, and used the computational ligand identification algorithm DOCK to identify in compuo enzyme inhibitors including oxalic bis(2-hydroxy-1-naphthylmethylene)hydrazide (**1**) [Ring, C. S.; Sun, E.; McKerow, J. H.; Lee, G.; Rosenthal, P. J., Kuntz, I. D.; Cohen, F. E., *Proc. Natl Acad. Sci. U.S.A.* **1993**, *90*, 3583]. Compound **1** inhibits falcipain (IC_{50} 6 μM) and the organism in vitro as judged by hypoxanthine uptake (IC_{50} 7 μM). Following this lead, to date, we have identified potent bis arylacylhydrazides (IC_{50} 150 nM) and chalcones (IC_{50} 200 nM) that are active against both chloroquine-sensitive and chloroquine-resistant strains of malaria. In a second example, cruzain, the crystallographically determined structure of a papain-like cysteine protease, resolved to 2.35 Å, was available. Aided by DOCK, we have identified a family of bis-arylacylhydrazides that are potent inhibitors of cruzain (IC_{50} 600 μM). These compounds represent useful leads for pharmaceutical development over strict enzyme inhibition criteria in a structure-based design program. Copyright © 1996 Elsevier Science Ltd

Introduction

More and more successful examples of the design of new ligands based on knowledge of target protein structure have been reported. These include design of noncovalent antiparasitic agents,[1–3] rational design of sialidase-based inhibitors of influenza virus replication,[4] design of nonpeptide cyclic ureas as HIV protease inhibitors,[5] structure-based discovery of inhibitors of thymidylate synthase,[6] and structure-based design of inhibitors of purine nucleoside phosphorylase.[7–10] Most structure-based drug design relies on X-ray crystallography/NMR spectroscopy[11] to obtain the appropriate structures to identify new leads and guide lead optimization. Our work on the structure-based drug design of parasitic protease inhibitors has relied upon X-ray crystal structures when available (e.g., cruzain[12,13] for anti-Chagas disease agents), but has relied on a homology-based model structure[1–3] if neither X-ray nor NMR data are available (e.g., falcipain for antimalarials) to generate leads and guide our lead optimization.

Proteases are involved in many important biological processes including protein turnover, blood coagulation, complement activation,[14] hormone processing,[15] and cancer cell invasion.[16] Thus, they are frequently chosen as targets for drug design and discovery. A potential strategy for the treatment of diseases caused by parasites is the design of compounds which selectively inhibit enzymes that are pivotal for survival of the parasite within the host that are part of biochemical pathways that are specific to the parasite. Parasite proteases are attractive target enzymes because of their roles in replication, metabolism, survival and pathology.[17]

In the most simple terms, structure-based drug design methods identify favorable and unfavorable interactions between a potential inhibitor and target receptor and maximize the beneficial interactions to increase binding affinity. Although X-ray crystallography continues to be the source of high-resolution information about protein structures, considerable delays often exist between determining the sequence of a protein and solving its structure. Difficulties in protein expression and more commonly in protein crystallization are often responsible for such delays. Currently, no general method exists to predict tertiary structure from amino acid sequences. However, when a protein target is relatively highly homologous to another protein or group of proteins of known structure, a sensible model structure can be proposed.[18]

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The World Health Organization estimates that 280 million people are infected with malaria[19] and 1–2 million deaths are reported annually.[20] While various classes of antimalarial agents are available, chloroquine and its derivatives remain the mainstay of therapy against malaria. Unfortunately, the emergence of malarial parasite strains resistant to chloroquine has eroded its efficacy.[21] This increases the urgency of the search for novel and cost-effective agents to treat chloroquine-resistant malaria.

Chagas disease is caused by *Trypanosoma cruzi*, a protozoan parasite that afflicts more than 24 million people in South and Central America. It is the leading cause of heart failure in many Latin American countries. Currently, there is no satisfactory treatment for this parasitic infection. Cruzain, the major cysteine protease present in *T. cruzi*, is pivotal for the development and survival of the parasite within the host cells. This makes the enzyme a target for potential trypanocidal drugs.

In our previous reports,[1–3] we described a structure-based approach to inhibitor design for antimalarial drug development using models of falcipain, a malaria trophozoite cysteine protease structure. Here, we summarize our progress to date in the structure-based drug design of parasitic protease inhibitors.

Results and Discussion

Lead discovery and optimization

A structure for the malaria cysteine protease, falcipain, was proposed using the X-ray structures of papain and actinidin, two cysteine proteases from plant sources, as a basis for homology modeling.[3] Falcipain has 33% sequence identity with both papain and actinidin. Moreover, ~60% of the conserved sequence centers around the active site regions. The homology-based model of the enzyme provides the template and the DOCK[22] algorithm calculates a set of spheres with approximately atom sized radii to fill the active site cleft. Within DOCK, the quality of a given compound's fit into the binding cleft can be evaluated based on its shape complementarity (contact score) or molecular mechanics interaction energy (AMBER force-field score).

The model structure was then used as a template for a DOCK search of the Fine Chemicals Directory of commercially available small molecules for putative ligands (the Fine Chemicals Directory distributed by Molecular Design Limited Information System, San Leandro, California, is currently known as the Available Chemical Directory). When searching a database of compounds, DOCK examines only the 'best' orientation of the small molecule within the binding cleft (DOCK database screening mode). When a single compound is studied, multiple possible binding modes can also be examined (DOCK single mode). Of course, the initial orientation of the compound is dictated in part by the irregular lattice of sphere centers identified originally. To overcome some of the scoring distortion that this bias could impart, a rigid body minimization algorithm has been developed to move the ligand within the binding cleft and optimize the shape or forcefield scores.[23] Compound **1** [oxalic *bis*((2-hydroxy-1-naphthylmethylene)hydrazide)] was selected based on its score for shape complementarity. Thirty-one compounds were finally tested and a lead compound **1** was identified as the best inhibitor of the protease. The IC_{50} value for enzyme inhibition against the substrate benzyloxycarbonyl-Phe-Arg-(7-amino-4-methylcoumarin) was 6 μM.[3] More importantly, this compound inhibits the growth of parasites in culture. Malaria lacks some of the enzymes required for *de novo* purine biosynthesis and thus depends on purine salvage pathways for DNA replication. Compound 1 inhibits parasite growth as judged by its ability to block hypoxanthine uptake, with an apparent IC_{50} value of 7 μM.3

Compound **1** fits a model of the active site of the malarial cysteine protease as shown in Figure 1.[1–3] The DOCK[22] program placed this lead compound **1** into the enzyme's active site, presumably filling three of the substrate side-chain specificity pockets (subsites S_2, S_1' and to a lesser extent S_1). Beginning with compound 1 as shown in Scheme 1, the following chemical modifications were made in an attempt to identify more active agents: (i) The length of the backbone linking the aromatic rings of **1** was shortened via the construction of asymmetric acylhydrazides, which could have less conformational heterogeneity than the symmetric hydrazides, yet still could fill at least two of the three putative subsites (**5** in Table 1). Compounds can be constructed by attaching a third aryl group to the center aromatic moiety to fill all three putative subsites (**6** in Table 1). (ii) Heterocyclic acylhydrazides were generated by incorporating nitrogen atoms into aromatic rings both to improve water solubility of the compounds and potentially to enhance electrostatic interactions with His67 in the S_2 site. (iii) To increase the chemical/metabolic stability of the compounds, a four-atom hydrazide linker was replaced with a three-atom α,β-unsaturated ketone bridge (**7** and **8** in Table 1). (iv) Naphthalene, quinoline or isoquinoline rings were exchanged for substituted phenyl rings on both acylhydrazide and α,β-unsaturated ketone linkers to explore the effective size and electronic character of the putative subsite specificity pocket.

Chemistry, antiparasitic activity and inhibition specificity

Since cost of production is a critically important consideration if the resulting antimalarials and other antiparasitic agents are ever to be developed into therapeutic agents for the world's developing countries, one of our guidelines for the development of antiparasitic agents is that these compounds should be inexpensive to produce. Hence, we developed relatively simple chemistry to prepare both arylacylhydrazide and chalcone derivatives. For both series, the final step is the condensation of an aldehyde with either acylhydrazines via imine formation[2] or substituted methylketones

via a Claisen–Schmidt condensation.[1] Since there are a variety of commercially available aldehydes and methylketones as starting materials, a large number of target compounds can be produced relatively inexpensively. For preparation of the key acylhydrazine intermediates, a published procedure was followed.[2]

After some 400 bis arylacylhydrazide and chalcone derivatives were synthesized, they were screened against three different antiparasitic screening systems. Antimalarial activities were evaluated based on an assay of parasitemia of red blood cells quantitated by a fluorescence-activated cell sorter (FACS) analysis[24] and the more classical assay of metabolic viability, hypoxanthine uptake,[1] described below.

For the FACS analysis, synchronized trophozoite-stage parasites were cultured in human blood at various inhibitor concentrations. The parasites were allowed to mature, the host cell was lysed and parasite invasion of

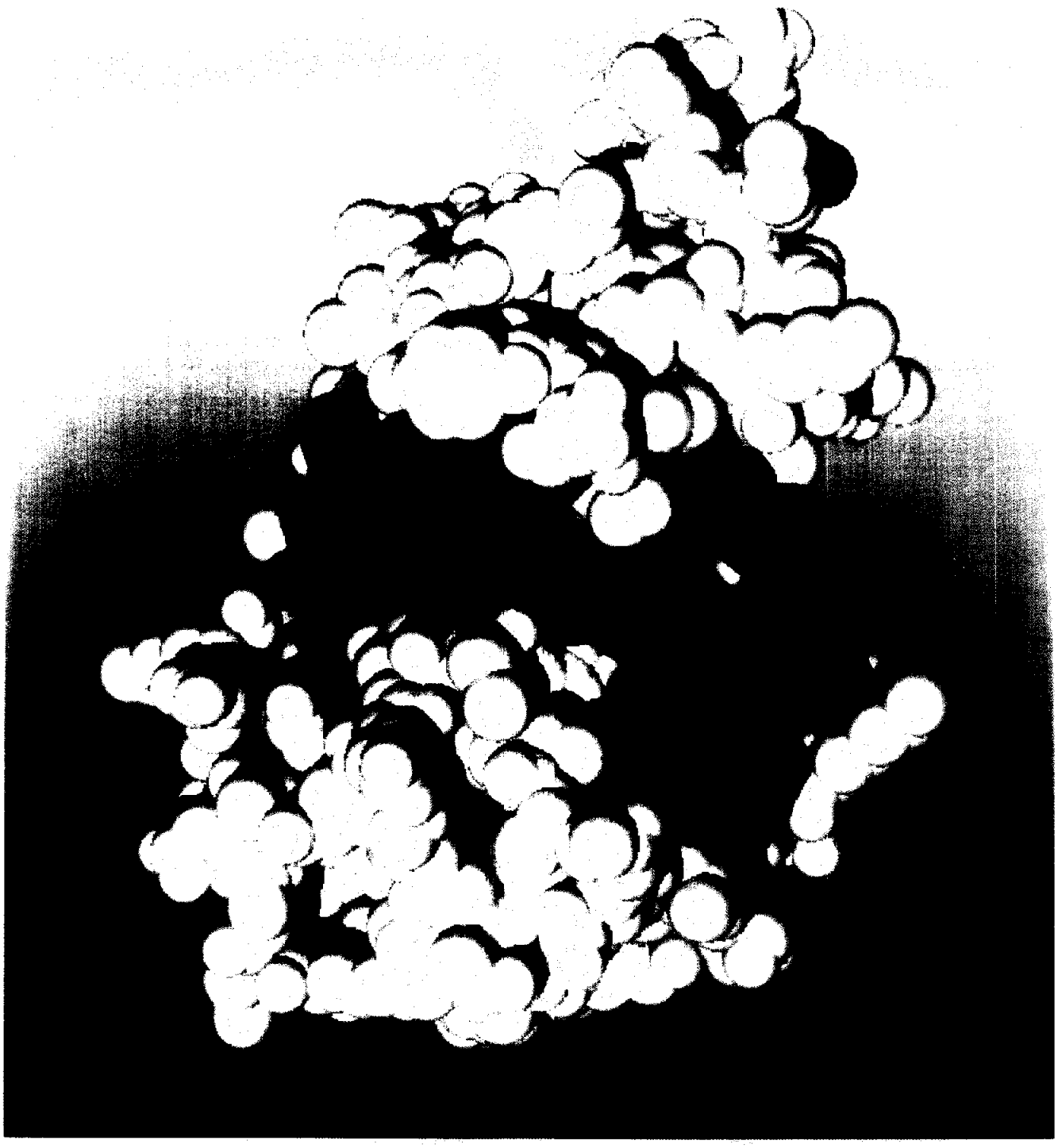
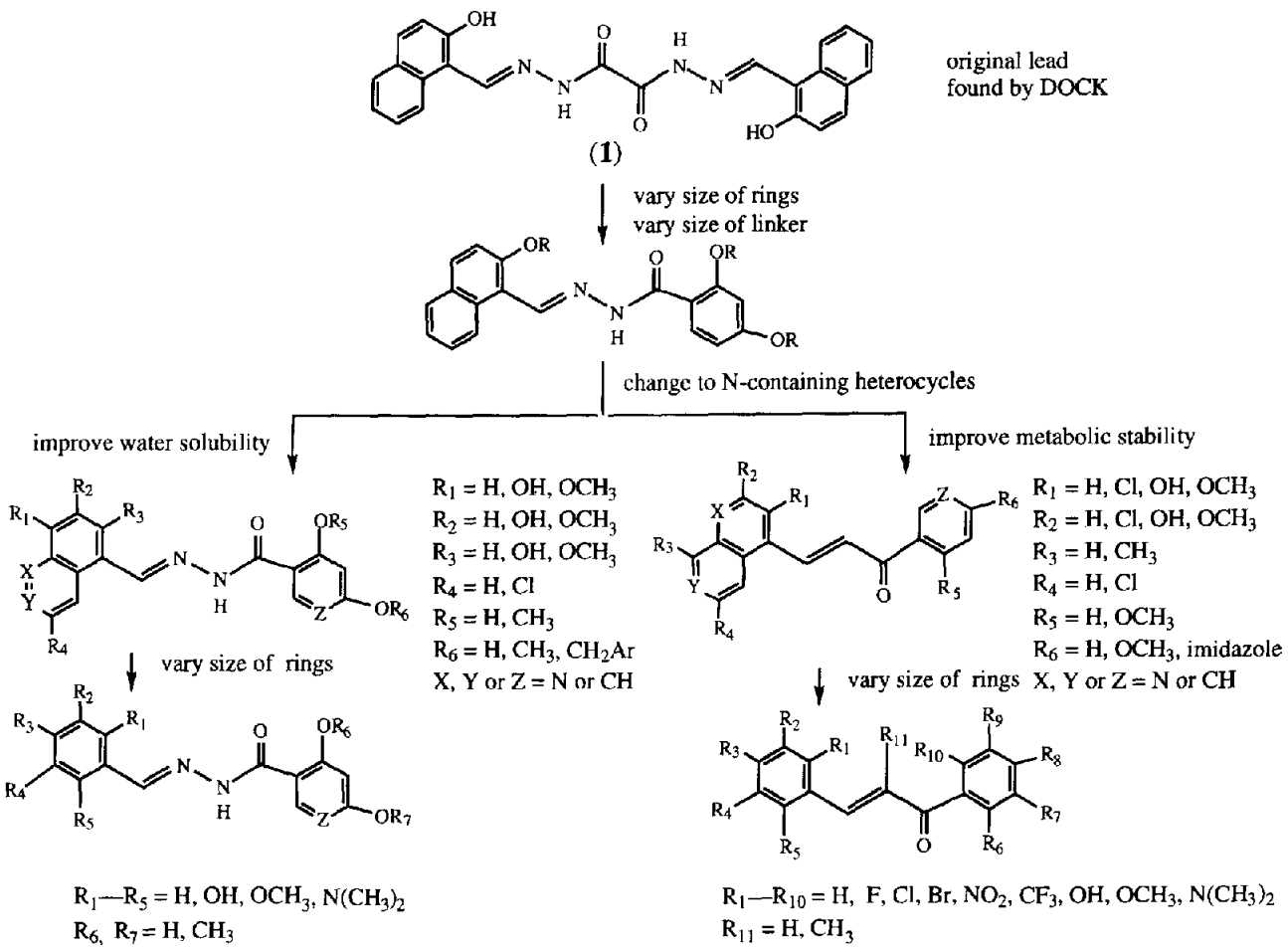


Figure 1. A putative binding orientation of the 'lead' compound (**1**) bound to the active site of the malarial cysteine protease. Key residues and the binding subsites of the protease are colored as cyan (S_1' site), yellow (S_1/catalytic site), and purple (S_2/S_3 site). For the lead compound, carbon is shown in green, oxygen in red, and nitrogen in blue.

fresh red blood cells was investigated. Using propidium iodide to stain DNA, the FACS can discriminate between infected and uninfected cells and between stages of intraerythrocytic parasite development as only infected red blood cells contain DNA.[24] The hypoxanthine uptake system assessed the intrinsic antimalarial activity in vitro against the erythrocytic asexual life cycle (blood schizontocides). Two *Plasmodium falciparum* clones, CDC/Indochina III (W2) and CDC/Sierra Leone I (D6),[25] were used for all antimalarial assays. W2 is resistant to chloroquine, quinine, and pyrimethamine and susceptible to mefloquine. D6 is resistant to mefloquine and susceptible to chloroquine, quinine and pyrimethamine. The resistance indexes are defined as ratios of the IC_{50} of a compound against W2 to the IC_{50} of the same compound against D6. This index is used as a factor to evaluate whether or not novel antimalarials are potential agents against chloroquine-resistant parasites. Chloroquine and mefloquine were used as controls in the assays. The third screening system involved the assay of two cysteine proteases, cruzain and cathepsin B.[1] Enzymatic activity of these two proteases was measured by following the cleavage of fluorogenic substrates.[1]

Structure–activity relationships (SAR) for some bis arylacylhydrazide and chalcone derivatives were reported previously.[1,2] Here, we summarize antiparasitic activity and enzyme inhibition of a group of hydrazide and chalcone derivatives. As shown in Table 1, the lead compound **1** showed little specificity in both antimalarial activity and enzyme inhibition. Cathepsin B is a mammalian cysteine protease from bovine spleen with 87% sequence identity to a human cathepsin B and papain is a cysteine protease from papaya. For comparison, inhibition activities of both cathepsin B and papain by hydrazide and chalcone derivatives are included in Table 1. Compound **2** is a more specific inhibitor of cruzain, with an IC_{50} value of 600 nM, than the rest of the compounds shown in Table 1. Compound **5** is the best in vitro antimalarial found in the acylhydrazide series with an IC_{50} value of 150 nM. The most noteworthy example is compound **6**. The IC_{50} value as an antimalarial is 450 nM while the IC_{50} value as a cathepsin B inhibitor is at least 400-fold higher (Table 1). This suggests that compound **6** is a more specific inhibitor of the malarial cysteine protease and will be less likely to be toxic to the host. Compound **7** is the best in vitro antimalarial compound found in the chalcone series to date with an IC_{50} value of ~200 nM.

The differences in inhibitory specificity shown above may stem from the fact that the active site residues of these cysteine proteases are quite different, as shown



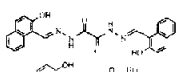
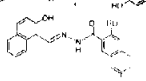
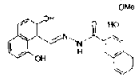
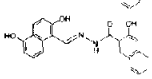
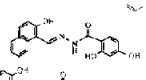
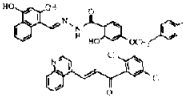
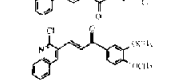
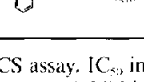
Scheme 1. 'Lead' optimization by chemical modification.

in Table 2, especially the residues from the S_2/S_3 site and part of the S_1/catalytic site. The most likely binding orientations of inhibitors involve extensive interactions between inhibitor molecules and the three binding subsites. The highly conserved S_1' and catalytic binding subsites provide basic interactions for binding, while the diverse residue types in the S_2/S_3 binding subsite among different proteases result in binding specificity. A thorough discussion of structural implications of binding specificity will be presented in a separate manuscript.[13]

Conclusions

Structure-based drug design typically depends upon the experimental determination of the target structure by either X-ray crystallography or NMR spectroscopy. We have circumvented this step and relied exclusively on the amino acid sequence homology between the malaria enzyme and other cysteine proteases of known structure to support our antimalarial program. A homology based model of the malaria enzyme served as the template for a computer-based ligand docking calculation that identified a useful lead compound for a group of cysteine proteases in our antiparasitic program. Lead optimization was achieved by a combined approach of computational and synthetic analysis. Derivatives of the lead were first optimized for fit using the computer docking program, and then numerous candidate compounds were synthesized and tested experimentally. Despite the lack of a detailed experimental structure of the target enzyme or the enzyme–inhibitor complex, we have been able to identify compounds with increased potency. To date, we have identified a potent bis arylacylhydrazide, **5** (IC_{50} 150 nM), and a chalcone, **7** (IC_{50} ~200 nM), that are active against both chloroquine-sensitive and chloroquine-resistant strains of malaria. Due to the ~30% sequence identity between cruzain and falcipain, a subgroup of compounds generated in the malaria project (selected using DOCK as a guide) were also screened against cruzain. The best cruzain inhibitor so far, hydrazide **2**, had an IC_{50} value of 600 nM.

Table 1. Antiparasitic activity and enzyme inhibition of some hydrazide and chalcone derivatives

Compds	Structure	Antimalarial activity	Cruzain[b]	Cathepsin B[b]	Papain[b]
1		7[a, ref 1]	0 (1) 91 (20)	22 (1) 78 (20)	19 (1) 74 (20)
2		>10[d]	0.6[f]	20[f]	50[f]
3		ND[c]	0.7[f]	20[f]	3[f]
4		ND	1.0[f]	30[f]	20[f]
5		0.150[e, ref 2]	ND	ND	ND
6		0.450[e, ref 2]	2.0[f]	200[f]	ND
7		0.230[d, ref 1] 0.190[e, ref 1]	ND	ND	ND
8		0.510[d, ref 1] 0.720[e, ref 1]	0 (1)	3 (1)	6 (1)

[a] FACS assay. IC_{50} in μM.
[b] % Enzyme inhibition (μM inhibitor).
[c] ND: not determined.
[d] Hypoxanthine uptake assay. IC_{50} of W2 (chloroquine-resistance strain) in μM.
[e] Hypoxanthine uptake assay. IC_{50} of D6 (chloroquine-sensitive strain) in μM.
[f] IC_{50} In μM.

Experimental

Melting points were measured on a Thomas–Hoover Unimelt apparatus and are uncorrected. TLC (silica gel 60 GF254, Merck, Darmstadt) was used to monitor reactions and check product homogeneity. NMR spectra at 300 MHz for ^1H and 75 MHz for ^{13}C (tetramethylsilane as internal standard) were recorded on a General Electric QE-300 spectrometer. Chemical ionization mass spectrometry (CIMS) spectra were obtained at the UCSF Mass Spectrometry Facility, A. L. Burlingame, Director. Elemental analyses were performed by the University of California, Berkeley, Microanalytical Laboratory and were within ±0.4% of the theoretical values. All starting materials were purchased from Aldrich Chemical Company, Inc.

General procedure for condensation of aldehyde with hydrazine (Method A for 2–6)

To a solution of the aldehyde (1 mmol) in methanol (20 mL) was added the corresponding acylhydrazine (1 mmol). The resulting mixture was heated at reflux at 65 °C for 3 h. In most cases, a precipitate was observed after 10 min. The precipitate was filtered, washed with

Table 2. Active site residues of cysteine proteases

	S_1'		S_1/catalytic site					S_2/S_3				
Proteases	19	177	175	25	159	67	68	69	133	160	205	207
Papain	Gln	Trp	Asn	Cys	His	Tyr	Pro	Trp	Val	Ala	Ser	Phe
Actinidin	—[a]	—	—	—	—	—	Ile	Thr	Ala	—	Met	Ser
Falcipain	—	—	—	—	—	His	—	Phe	Asn	Ser	Glu	—
Cruzain	—	—	—	—	—	Leu	Met	Asn	Ala	Gly	Glu	Ser
Cathepsin B	—	—	—	—	—	Glu	—	Ser	Ala	—	Glu	Val

[a] Same residue type as papain.

hot methanol (50 mL) and dried under vacuum to give a solid (usually yellow). If needed, additional purification was performed by recrystallization using appropriate solvents.

Preparation of acylhydrazines from the corresponding acid or ester (Method A1)

A mixture of the acid (10 mmol) and concd H_2SO_4 (5 mL) in MeOH (25 mL) was heated at reflux for 12–24 h. The mixture was poured onto ice (100 mL). The resulting precipitates were collected, washed with H_2O and recrystallized from EtOH:H_2O to give the pure methyl ester. The methyl ester was then dissolved in EtOH (80 mL) and treated with hydrazine monohydrate (5.01 g, 100.0 mmol). The resulting mixture was stirred overnight at 20 °C and then concentrated to give the corresponding acylhydrazine, which was further purified by recrystallization from EtOH/H_2O.

Compound **1** [oxalic bis(2-hydroxy-1-naphthylmethylene)hydrazide] was reported previously.[2] Chalcone derivatives **7** [1-(2,5-dichlorophenyl)-3-(4-quinolinyl)-2-propen-1-one] and **8** [1-(3,4-dimethoxyphenyl)-3-(3-(2-chloroquinolinyl))-2-propen-1-one] were prepared via a Claisen-Schmidt condensation also described previously.[1]

2′-Hydroxy-6′-methoxynaphthylacyl-(2-hydroxy-1-naphthylmethylene)hydrazide (2). A 70% yield; mp >280 °C; ^{1}H NMR (DMSO-d_6): δ 12.84 (s, 1 H), 12.20 (br s, 1 H), 11.20 (br s, 1 H), 9.59 (s, 1 H), 8.46 (s, 1 H), 8.36 (d, 1 H, J=8.4 Hz), 7.96 (d, 1 H, J=9.0 Hz), 7.91 (d, 1 H, J=8.1 Hz), 7.74 (d, 1 H, J=9.0 Hz), 7.63 (t, 1 H, J=.6 Hz), 7.43 (t, 1 H, J=7.5 Hz), 7.28 (d, 1 H, J=9.0 Hz), 7.22 (dd, 1 H, J=2.0, 9.1 Hz), 3.90 (s, 3 H); ^{13}C NMR (DMSO-d_6): δ 163.08, 158.14, 155.73, 152.16, 147.48, 132.89, 131.71, 131.48, 129.25, 128.93, 127.81, 127.76, 127.72, 127.44, 123.56, 121.31, 121.00, 119.94, 118.21, 110.94, 108.65, 106.45, 55.17; CIMS: m/z (MH^+) 387.3. Anal. ($C_{23}H_{18}N_2O_4$): calcd: C, 71.49; H, 4.70; N, 7.25. Found: C, 71.09; H, 4.84; N, 7.23%.

2′-Hydroxynaphthylacyl-(2,8-dihydroxy-1-naphthylmethylene)hydrazide (3). A 88% yield; mp 274 °C (dec); ^{1}H NMR (DMSO-d_6): δ 14.03 (s, 1 H), 12.54 (br s, 1 H), 11.31 (br s, 1 H), 10.47 (s, 1 H), 10.37 (s, 1 H), 8.53 (s, 1 H), 7.96 (d, 1 H, J=8.1 Hz), 7.87 (d, 1 H, J =9.0 Hz), 7.80 (d, 1 H, J=8.4 Hz), 7.54 (t, 1 H, J =7.5 Hz), 7.39 (m, 3 H), 7.22 (m, 2 H), 7.08 (d, 1 H, J =7.2 Hz); ^{13}C NMR (DMSO-d_6): δ 163.80, 159.39, 154.34, 153.30, 153.02, 135.93, 133.51, 130.26, 130.06, 128.66, 128.28, 126.73, 125.91, 123.82, 123.71, 121.72, 120.50, 119.80, 119.43, 112.51, 110.67, 108.87; CIMS: m/z (MH^-) 373.3. Anal. ($C_{22}H_{16}N_2O_4 \cdot 0.2H_2O$): calcd: C, 70.28; H, 4.40; N, 7.45. Found: C, 70.45; H, 4.58; N, 7.40%.

2′-Hydroxynaphthylacyl-(2,5-dihydroxy-1-naphthylmethylene)hydrazide (4). A 74% yield; mp >280 °C; ^{1}H NMR (DMSO-d_6): δ 12.81 (s, 1 H), 12.23 (s, 1 H), 11.29 (s, 1 H), 10.22 (s, 1 H), 9.52 (s, 1 H), 8.52 (s, 1 H), 8.20 (d, 1 H, J=9.2 Hz), 7.59 (d, 1 H, J=8.1 Hz), 7.80 (d, 1 H, J=8.2 Hz), 7.71 (d, 1 H, J=8.6 Hz), 7.54 (t, 1 H, J=7.5 Hz), 7.41 (m, 3 H), 7.16 (d, 1 H, J=9.2 Hz), 6.80 (d, 1 H, J=7.5 Hz); ^{13}C NMR (DMSO-d_6) δ 163.04, 158.38, 154.10, 153.89, 148.09, 135.93, 133.39, 130.52, 128.66, 128.52, 128.32, 127.01, 126.78, 125.84, 123.85, 119.84, 118.93, 117.08, 111.40, 110.60, 108.29, 106.46; CIMS: m/z (MH^+) 373.2. Anal. ($C_{22}H_{16}N_2O_4 \cdot 0.5H_2O$): calcd: C, 69.28; H, 4.49; N, 7.35. Found: C, 69.35; H, 4.54; N, 7.27%.

2′,4′-Dihydroxyphenylacyl-(2-hydroxy-1-naphthylmethylene)hydrazide (5). A 81% yield; mp >280 °C; ^{1}H NMR (DMSO-d_6): δ 12.83 (s, 1 H), 12.18 (br s, 1 H), 12.01 (br s, 1 H), 10.35 (br s, 1 H), 9.54 (s, 1 H), 8.32 (d, 1 H, J=8.6 Hz), 7.88 (m, 3 H), 7.62 (t, 1 H, J=7.6 Hz), 7.42 (t, 1 H, J=7.4 Hz), 7.26 (d, 1 H, J=8.9 Hz), 6.47 (d, 1 H, J=8.8 Hz), 6.43 (s, 1 H); ^{13}C NMR (DMSO-d_6): δ 164.58, 162.94, 161.90, 158.02, 146.90, 132.72, 131.67, 129.96, 128.94, 127.80, 127.71, 123.54, 120.87, 178.92, 108.66, 107.78, 106.14, 102.93; CIMS: m/z (MH^+) 323.1. Anal. ($C_{18}H_{14}N_2O_4$): calcd: C, 67.08; H, 4.38; N, 8.69. Found: C, 67.04; H, 4.53; N, 8.91%.

2′-Hydroxy-4′-(4-nitrobenzyloxy)phenyacyl-(2,4-dihydroxy-1-naphthylmethylene)hydrazide (6). A 91% yield; mp >270 °C; ^{1}H NMR (DMSO-d_6): δ 12.82 (s, 1 H), 12.46 (br s, 1 H), 11.90 (br s, 1 H), 11.03 (br s, 1 H), 9.39 (s, 1 H), 8.25 (d, 1 H, J=8.1 Hz), 8.17 (d, 1 H, J=8.6 Hz), 8.10 (d, 1 H, J=8.3 Hz), 7.91 (d, 1 H, J=8.8 Hz), 7.71 (d, 1 H, J=8.2 Hz), 7.58 (t, 1 H, J =7.6 Hz), 7.34 (t, 1 H, J=7.5 Hz), 6.91 (d, 1 H, J=8.8 Hz), 6.61 (s, 1 H), 6.60 (s, 1 H), 5.33 (s, 2 H); ^{13}C NMR (DMSO-d_6): δ 164.23, 162.47, 162.10, 160.37, 157.62, 147.94, 147.07, 144.41, 132.92, 129.41, 128.27, 128.27, 123.66, 123.66, 122.93, 122.50, 120.67, 120.28, 107.75, 107.10, 102.39, 101.34, 100.46, 68.23; CIMS: m/z (MH^+) 474.2. Anal. ($C_{25}H_{19}N_3O_7 \cdot 2/3H_2O$): calcd: C, 61.85; H, 4.12; N, 8.66. Found: C, 61.77; H, 4.33; N, 8.67%.

Biological assays

The previously published FACS assay protocol was followed for in vitro antimalarial testing.[24] Hypoxanthine uptake and enzyme (cruzain, cathepsin B and papain) inhibition procedures were also published previously.[1]

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References

1. Li, R.; Chen, X.; Gong, B.; Dominguez, J. N.; Davidson, E.; Kurzban, G.; Miller, R. E.; Nuzum, E. O.; Rosenthal, P. J.; McKerrow, J. H.; Kenyon, G. L.; Cohen, F. E. *J. Med. Chem.* **1995**, *38*, 5031.

2. Li, Z.; Chen, X.; Davidson, E.; Zwang, O.; Mendis, C.; Ring, C. S.; Roush, W. R.; Fegley, G.; Li, R.; Rosenthal, P. J.; Lee, G. K.; Kenyon, G. L.; Kuntz, I. D.; Cohen, F. E. *Chem. Biol.* **1994**, *1*, 31.

3. Ring, C. S.; Sun, E.; McKerrow, J. H.; Lee, G.; Rosenthal, P. J.; Kuntz, I. D.; Cohen, F. E. *Proc. Natl Acad. Sci. U.S.A.* **1993**, *90*, 3583.

4. von Itzstein, M.; Wu, W.-Y.; Kok, G. B.; Pegg, M. S.; Dyason, J. C.; Jin, B.; Phan, T. V.; Smythe, M. L.; White, H. F.; Oliver, S. W.; Colman, P. M.; Vargheses, J. N.; Ryan, D. M.; Woods, J. M.; Bethell, R. C.; Hotham, V. J.; Cameron, J. M.; Penn, C. R. *Nature (London)* **1993**, *363*, 418.

5. Lam, P. Y. S.; Jadhav, P. K.; Eyermann, C. J.; Hodge, C. N.; Ru, Y.; Bacheler, L. T.; Meek, J. L.; Otto, M. J.; Rayner, M. M.; Wong, Y. N.; Chang, C.-H.; Weber, P. C.; Jackson, D. A.; Sharpe, T. R.; Erickson-Viitanen, S. *Science* **1994**, *263*, 380.

6. Shoichet, B. K.; Stroud, R. M.; Santi, D. V.; Kuntz, I. D.; Perry, K. M. *Science* **1993**, *259*, 1445.

7. Montgomery, J. A.; Niwas, S.; Rose J. D.; Secrist, III, J. A.; Babu, Y. S.; Bugg, C. E.; Erion, M. D.; Guida, W. C.; Ealick, S. E. *J. Med. Chem.* **1993**, *36*, 55.

8. Secrist, III, J. A.; Niwas, S.; Rose, J. D.; Babu, Y. S.; Bugg, C. E.; Erion, M. D.; Guida, W. C.; Ealick, S. E.; Montgomery, J. A. *J. Med. Chem.* **1993**, *36*, 1847.

9. Erion, M. D.; Niwas, S.; Rose J. D.; Ananthan, S.; Allen, M.; Secrist, III, J. A.; Babu, Y. S.; Bugg, C. E.; Guida, W. C.; Ealick, S. E.; Montgomery, J. A. *J. Med. Chem.* **1993**, *36*, 3771.

10. Guida, W. C.; Elliott, R. D.; Thomas, H. J.; Secrist, III, J. A.; Babu, Y. S.; Bugg, C. E.; Erion, M. D.; Ealick, S. E.; Montgomery, J. A. *J. Med. Chem.* **1994**, *37*, 1109.

11. Fesik, S. W. *J. Biomol. NMR* **1993**, *3*, 261.

12. McGrath, M. E.; Eakin, A. E.; Engel, J. C.; McKerrow, J. H.; Craik, C. S.; Fletterick, R. J. *J. Mol. Biol.* **1995**, *247*, 251.

13. Chen, X.; Li, R.; Gong, B.; Davidson, E.; Dominguez, J. N.; McKerrow, J. H.; Kenyon, G. L.; Cohen, F. E. manuscript in preparation.

14. Stryer, L. *Biochemistry*; Freeman: New York, 1988.

15. Thomas, L.; Leduc, R.; Thorne, B. A.; Smeekens, S. P.; Steiner, D. F.; Thomas, G. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 5297.

16. Cohen, R. L.; Xi, X. P.; Crowley, C. W.; Lucas, B. K.; Levinson, A. D.; Shuman, M. A. *Blood* **1991**, *72*, 479.

17. McKerrow, J. H.; Sun, E.; Rosenthal, P. J.; Bouvier, J. *Annu. Rev. Microbiol.* **1993**, *47*, 821.

18. Ring, C. S.; Cohen, F. E. *FASEB J.* **1993**, *7*, 783.

19. Gibbons, A. *Science* **1992**, *256*, 1135.

20. Walsh, J.A. *Ann. N. Y. Acad. Sci.* **1989**, *569*, 1.

21. World Health Organization Malaria Action Program. *Trans. R. Trop. Med. Hyg.* **1986**, *80*, 1.

22. Kuntz, I. D. *Science* **1992**, *257*, 1078; DOCK V 3.0 was used in all initial phases of this work. The current release is DOCK V 3.5. Contact I.D. Kuntz for distribution information.

23. Meng, E. C.; Gschwend, D. A.; Blaney, J. M.; Kuntz, I. D. *Proteins* **1993**, *17*, 266.

24. Clark, D. L.; Chrisey, L. A.; Campbell, F. R.; Davidson, E. A. *Molec. Biochem. Parasitol.* **1994**, *63*, 129.

25. Oduola, A. M.; Weatherly, N. F.; Bowdre, J. H.; Desjardins, R. E. *In Vitro. Exp. Parasitol.* **1988**, *66*, 86.

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