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Identification of Novel Falcipain-2 Inhibitors as Potential Antimalarial Agents through Structure-Based Virtual Screening

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The SPECS database was screened against falcipain-2 with two different docking methods to identify structurally diverse nonpeptidic inhibitors. Twenty-eight nonpeptidic molecules among 81 compounds tested were identified as potential inhibitors of falcipain-2. One of the inhibitors exhibited in vitro activity with an IC_{50} value of 2.4 μM. Furthermore, the similarity analysis has demonstrated that it is feasible to find novel diverse falcipain-2 inhibitors with similar steric shape through virtual screening of large-scale chemical libraries.

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Introduction

Along with tuberculosis and AIDS,[a] malaria is one of the three most devastating infectious diseases,[1] affecting nearly 300−500 million people each year and killing 1.5−2.7 million of them, especially in the developing world.[2] Currently, no effective vaccine is available.[3] In the past two decades, the number of malaria cases has been growing steadily, mainly due to the development of drug resistance in the parasite,[4] in particular *Plasmodium falciparum*.[5,6] Thus, in many parts of the world, the parasite is now resistant against chloroquine, which was by far the most frequently used antimalaria drug for half a century.[7,8] Therefore, there is an increasing need to identify new targets and develop drugs aimed at these targets. Various potential biochemical targets have been proposed;[9,10] among these, the cysteine protease falcipain-2 from *P. falciparum* is an attractive and most promising target enzyme, which plays a key role in hemoglobin degradation in throphozoites.[11,12]

Numerous peptide-based falcipain-2 inhibitors, which tend to form covalent bonds with the thiolate of the catalytic cysteine and exhibit nanomolar IC_{50} values, have been identified, such as vinyl sulfones (irreversible),[13] ketone-based,[14] and aldehyde-based[15] (reversible) inhibitors. Obviously, it is desirable to design nonpeptidic inhibitors that would bind noncovalently to the target enzyme in order to minimize toxicity while retaining the potential for high in vivo activity and selectivity.[16] To our knowledge, only a few nonpeptidic inhibitors of falcipain-2 with IC_{50} values in the micromolar range have been reported so far,[16−23] most of which were designed based on homology models because the crystal structure for falcipain-2 had not yet been determined at the time of these studies. Recently, crystal structures for falcipain-2 have been reported,[24,25] and structural and functional properties of falcipain-2 were described in detail, which provided structural knowledge crucial for the design of novel antimalarial drugs.

Using virtual screening, Desai et al. recently identified more than 40 nonpeptidic cysteine protease inhibitors from the ChemBridge and ACD databases.[16,17] Here we report the successful application of two virtual screening programs, Glide[26,27] and GAsDock,[28] to search the SPECS database[29] (http://www.specs.net/) for novel potential falcipain-2 inhibitors. We also present data on the biological activity and experimental details for these inhibitors.

Experimental Section

Protein Preparation. The crystal structure of falcipain-2 from *Plasmodium falciparum*, previously determined by the group of one of us (RH),[30] was retrieved from the Protein Data Bank[30] (PDB entry: 2GHU). Amino acid residues located within 14 Å from the catalytic thiolate of Cys42 were defined as part of the binding site for docking studies. All crystallographic water molecules were removed from the coordinate set. Because two different virtual screening programs were used, different preparations were performed in the following sections.

Glide. Glide calculations were performed with Maestro v7.5 (Schrodinger, Inc.).[26,27] Hydrogen atoms and charges were added during a brief relaxation performed using the Protein Preparation module in Maestro with the "preparation and refinement" option, and a restrained partial minimization was terminated when the root-mean-square deviation (rmsd) reached a maximum value of 0.3 Å in order to relieve steric clashes. The grid-enclosing box was centered on the sulfur atom of Cys42 and defined so as to enclose residues located within 14 Å from the catalytic thiolate, and a scaling factor of 1.0 was set to van der Waals (VDW) radii of those receptor atoms with the partial atomic charge less than 0.25. In the docking process, standard-precision (SP) and extra-precision (XP) docking were respectively adopted to generate the minimized pose, and the Glide scoring function (G-Score) was used to select the final 30 poses for each ligand. The docking simulations were performed on a Dell Cluster server in parallel.

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[a] Abbreviations: AIDS, acquired immune deficiency syndrome; rmsd, root-mean-square deviation; VDW, van der Waals; SP, standard precision; XP, extra-precision; IMPGA, improved multipopulation genetic algorithm; GA, genetic algorithm; GSH, glutathione; GSSG, glutathione disulfide; DTT, dithiothreitol; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; FCFP, functional class fingerprints; USR, ultrafast shape recognition; SAR, structure−activity relationship; ITC, isothermal titration calorimetry.

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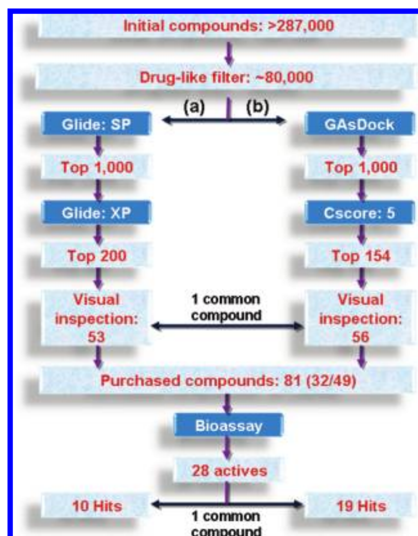

Figure 1. Schematic representation of the virtual screening approaches adopted.

GAsDock. GAsdock is a novel and fast flexible docking program, which employs an improved multipopulation genetic algorithm (IMPGA) based on information theory for the conformational search of the ligand.[28] First, hydrogen atoms and Kollman charges were assigned to the protein target using the standard Sybyl package (Tripos Inc., St. Louis, MO). To compute interaction energies, a three-dimensional grid was centered on the active-site Cys42. Energy scoring grids were obtained using an all-atom model and a distance-dependent dielectric function ($\varepsilon = 4r$) with a 10 Å cutoff. Database molecules were then docked into the protein active site. For the genetic algorithm (GA) run, the population size M was set to 6, with an individual size $N = 30$ in each population. The genetic algorithm parameters were defined as follows: tournament size 2 for selection, crossover probability $P_c = 0.85$, mutation probability $P_m = 0.1$, and replacement size $\eta = 1$ in each narrowed population. The GAsDock docking was performed on an Origin3800 supercomputer (450 MHz).

Virtual Screening. Virtual screening of large chemical databases is a successful approach for lead identification. However, different docking programs with various scoring functions are known to emphasize different aspects of ligands.[31] Here, two docking programs, Glide and GAsDock, with different scoring functions, were employed to identify leads for novel falcipain-2 inhibitors (Figure 1).

Chemistry. The purity of 28 hits that were essential to the conclusions drawn in the text were determined by HPLC on an Agilent 1200 series instrument equipped with a Diamonsil-C18 column (250 mm × 4.6 mm, 5 μm particle size) and a UV/vis detector setting at $\lambda = 254$ nm. All compounds were eluted with the solvent systems listed in Supporting Information Table S1 at a flow rate of 1 mL/min.

Enzyme Inhibition Assay. The purification and refolding of recombinant falcipain-2 was performed as described by Shenai et al.[11] IC_{50} values against falcipain-2 were determined as described previously.[32,33] Enzyme (30 nM) was incubated for 30 min at room temperature in 100 mM sodium acetate, pH 5.5, 10 mM DTT, with different concentrations of the inhibitors to be tested. Inhibitor solutions were prepared from stock in DMSO (maximum concentration of DMSO in the assay was 1%). After 30 min incubation, the substrate Z-Leu-Arg-AMC (benzyloxycarbonyl-Leu-Arg-7-amino-4-methylcoumarin) in the same buffer was added to a final concentration of 25 μM. The increase in fluorescence was monitored for 30 min at room temperature with an automated microtiter plate spectrofluorimeter (Molecular Devices, Flex station). IC_{50} values were determined from plots of percent activity over compound concentration using the GraphPad Prism software with three independent determinations (The IC_{50} values were determined if the inhibition rates of compounds at 10 μM were larger than 20%, and the maximum concentrations of the tested compounds were 50 μM.).

Table 1. Inhibitory Rates and Inhibitory Activities against Falcipain-2[a]

compd	inhibition rate (%)	IC_{50} (μM)[b]
1	53.1	2.4
2	71.4	5.8
3	56.5	6.4
4	41.4	10.9
5	48.5	13.2
6	38.3	17.1
7	29.4	29.7
8	20.3	54.2
9	36.6	~15
10	44	~15
11	25.2	20−50
12	28.9	20−50
13	27.9	20−50
14	28.2	20−50
15	36.9	20−50
16	26.9	20−50
17	29	20−50
18	25.9	20−50
19	21.3	> 50
20	20.2	> 50
21	20.2	> 50
22	20.2	> 50
23	23.4	> 50
24	21.9	> 50
25	20.8	> 50
26	21.8	> 50
27	25.1	> 50
28	21.9	> 50
E-64	97.4	0.017

[a] The inhibition rate (%) was calculated using the equation: [1 - ($F$460/$F$460 control)] × 100%. All values are the means of three independent determinations and the deviations are < 10% of the mean value. [b] IC_{50} values were determined from three separate experiments; each compound concentration was tested in triplicate. Attempts to determine IC_{50} values were made if the inhibition rate at 10 μM was larger than 20%.

Characterization of Interaction between Falcipain-2 and Inhibitors. Falcipain-2 (30 nM) was incubated with **E-64** (10 μM) or compound **1** (10 μM) at room temperature for 30 min to block its active site; a lack of activity against Z-Leu-Arg-AMC after this was confirmed with the spectrofluorometric assay described above. Then the samples were dialyzed in an optimized refolding buffer at 4 °C for 8 h and their activities were tested again.

The protein incubated with compound **1** was analyzed by gel permeation chromatography using a Sephadex G-75 column (Amersham Pharmacia) on an AKTA fast protein liquid chromatography system (Amersham Pharmacia). The column was equilibrated with 20 mM Tris, pH 7.5. Furthermore, the refolded protein was incubated with compound **1** at 37 °C for 30 min and then incubated with and without DTT at a final concentration of 100 mM at 37 °C for another 30 min. Subsequently, equal volumes of nonreducing SDS-PAGE sample buffer were added; the nonreduced sample was not boiled, whereas reduced samples were boiled for 10 min. The samples were resolved by 10% SDS-PAGE and visualized by Coomassie Blue staining.

Similarity Analysis. To measure the diversity of the hits, the Tanimoto similarity indices[34] for the compounds were calculated using SciTegic functional class fingerprints (FCFP_4)[35,36] in Pipeline Pilot 5.0. The FCFP fragments encode six generalized atom types, and FCFP_4 denotes the circular substructure based on atom functional descriptor.[36] The similarity metric containing the Tanimoto coefficient was calculated for the required fingerprint properties and the similarity values for

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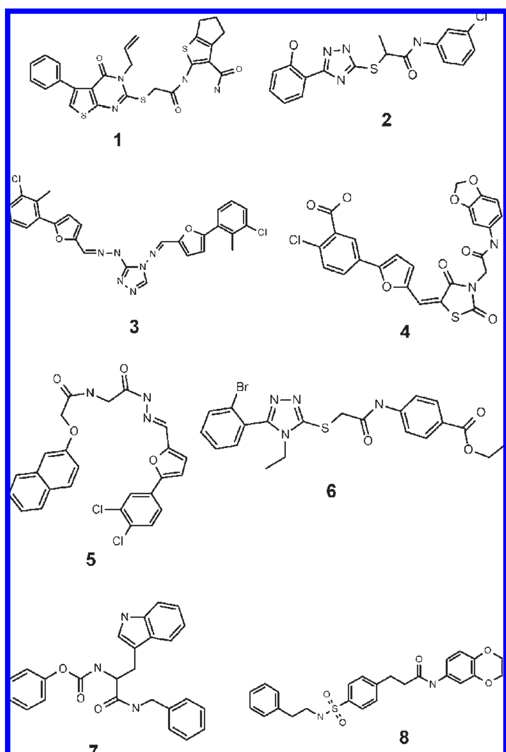

Figure 2. Structures of falcipain-2 inhibitors identified using virtual screening.

each hit molecule, and the average or the maximum similarity was also derived.

Furthermore, shape similarity of the hits was assessed using a fast computational method, Ultrafast Shape Recognition (USR), for rigid recognition of molecular shape, which is based on the observation that the shape of a molecule is uniquely determined by the relative positions of its atoms,[37,38] and these positions are in turn determined by the set of all interatom distances. The shape similarity of each pair of the docked conformations of corresponding hit molecules were analyzed by USR, and a correlation matrix was established according to the calculated pairwise normalized similarity score.

Results and Discussion

As presented in Figure 1, a total of 287000 compounds in the SPECS database were reduced to about 80000 using the druglike filter developed by Zheng et al.[39] These 80000 compounds were subsequently docked and ranked with the programs Glide and GAsDock in two parallel docking routes ((a) and (b) in Figure 1). From the (a) run, the top 1000 compounds were selected using Glide with SP docking for further accurate docking, which was carried out using Glide with XP docking. Lastly, the binding poses of the top 200 compounds were stored for visual inspection of the docking geometry according to the following criteria: (1) complementarity between the ligand and the hydrophobic S2 pocket of the protein; (2) formation of hydrogen bonds between the ligand and residues near the catalytic cysteine (Cys42). Finally, 53 compounds were selected to be purchased from the SPECS vendor. From the (b) run, the top 1000 compounds out of 80000 druglike compounds were selected using GAsDock and its energy score. The top 1000 compounds were further evaluated and ranked using the CSCORE module of the Sybyl package. A list of 154 compounds was then selected with the consensus score of 5. By visual analysis of the 154 docked poses, 98 compounds not conforming to the above-mentioned criteria were eliminated. Thus, 56 compounds were selected to be purchased from the SPECS vendor. There was only 1 common compound from the above independent routes. Of the total of 108 compounds so selected, only 81 could be procured and submitted to experimental evaluation, i.e., measurement of in vitro inhibition of falcipain-2.

The results of the biological evaluation are presented in Table 1. Out of 81 compounds procured, 28 compounds were found to be inhibitors of falcipain-2 with IC_{50} values ranging from 2.4 to 54.2 μM (Figure 2). Unfortunately, the IC_{50} values of some compounds (compounds **9**–**28**, Figure S1) could not be precisely determined due to their poor solubility. However, the results were very encouraging, with a success rate of 35% for our virtual screening approach (28 active compounds out of 81 tested). The hit rates of GAsDock and Glide are 38.8% (19/49) and 31.3% (10/32), respectively. The reason for the difference in performance might be that GAsDock, which employs the DOCK energy score, is more reliable for the apolar falcipain-2 active site compared with Chemscore-based GlideScore. To increase the hit rate, it is of crucial importance to first test whether a scoring function is able to distinguish active compounds from random compounds against a special target before screening a large chemical database.[40] Through the comparison of two alternative docking programs and the following analysis of the binding site, those scoring functions that perform well for apolar and hydrophobic active sites, such as the DOCK energy score and GoldScore,[16,17] outperform those that tend to perform well for polar binding sites, such as Chemscore and FlexX. This observation also provides some clues and knowledge for future screening and structural modification.

The predicted binding poses of inhibitors (compounds **1**–**28**) are shown in Figure 3. Most of them have similar interactions with the residues in the active site, and all the compounds effectively span and occupy the S2 and S1′ pockets with no significant interactions with the S1 pocket. In particular, compound **13** shows additional interactions with the S3 pocket (orange in Figure 3). Several interesting features of possible inhibitors can be derived from the SAR analysis of the docking hits: (1) it is obvious that there is a very large hydrophobic region in the S1′ subsite, and the inhibitors fit well into the S2 and S1′ subsites with VDW interactions and hydrophobic forces; (2) in particular, there are distinct hydrophobic interactions with Val152 (in the S1′ subsite) for compounds **1** and **2**, explaining why additional lipophilic substituents such as allyl or chloro seem to give better potency (Figure 4a,b); (3) in addition, some compounds are involved in $\pi-\pi$ stacking interactions between their aromatic system and Trp206 in the S1′ pocket, such as the phenyl group of compound **1** (Figure 4a); an aromatic group at the terminus (interacting with the S1′ pocket) appears to be necessary for good activity, such as in compounds **1**, **3**, **4**, **5**, **8**, and **9**; (4) some nonpolar residues with aliphatic side chains, such as Gly83, Ile85, and Ala235, form a hydrophobic cavity in S2 subsite in which the aliphatic groups at this terminus of all the hits were embedded; (5) the central saddle linker (connecting inhibitor moieties binding to S2 and S1′ subsites) in most of the hit molecules can form hydrogen bonds with the polar residues around the active-site Cys42, including Gly83, Asn173, and His174, or the ring nitrogen in Trp206 (Figures 4a,b).

For the 28 active compounds, the average pairwise Tanimoto similarity index was 0.25 (Supporting Information Figure S2 and Table S2), indicating that the compounds were chemically diverse. The maximum similarity was 0.82 for compound pairs (**4** and **17**, **11** and **19**), which share the same scaffolds. Moreover, the shapes of the 28 hits are very similar

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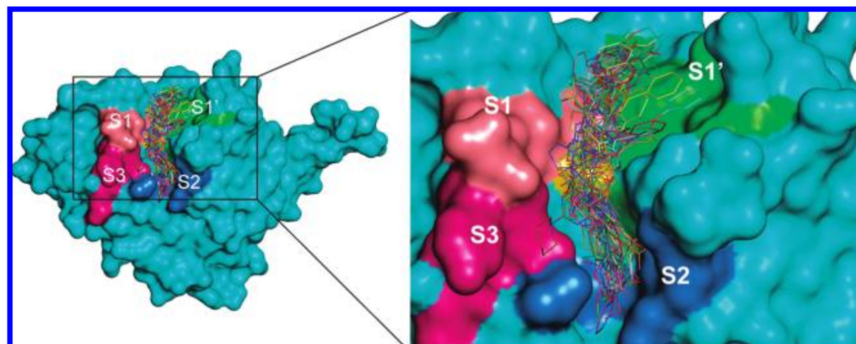

Figure 3. Superimposition of the docked conformations of inhibitors and enlarged view (right) in the active site of falcipain-2. The ligands are shown in line with the noncarbon atoms in standard colors, and hydrogen atoms have been omitted for clarity. The subsites are labeled S1, S1′, S2, and S3. The structure figures were prepared using PyMol (http://pymol.sourceforge.net/).

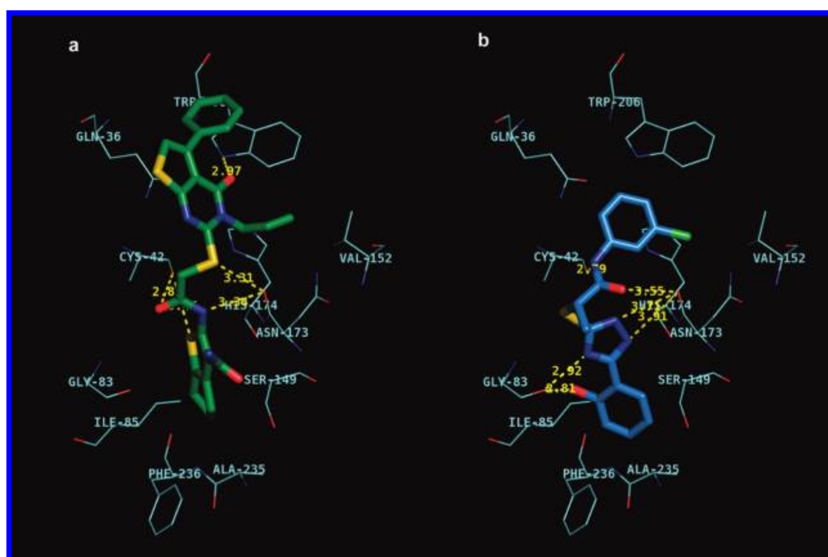

Figure 4. Binding poses for compounds **1** and **2**. (A) Proposed binding mode of compound **1** in the active site of falcipain-2 (in green). (B) Proposed binding mode of compound **2** in the active site of falcipain-2 (in sky-blue). The compounds are shown as sticks and noncarbon atoms are colored by atom types. Critical residues of the binding pocket are shown as lines in cyan. Hydrogen bonds are shown as dotted yellow lines with distance between donor and acceptor atoms.

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to each other (Supporting Information Table S3), with an average similarity score of 0.73, indicating that these compounds match very well in steric shape, further implying that the 28 hits have similar interactions with the residues in the binding site. The similarity analysis demonstrates that it is feasible to find novel diverse falcipain-2 inhibitors, especially novel scaffolds with similar steric shape, through virtual screening of large-scale chemical library.

To evaluate the interaction between falcipain-2 and our designed inhibitor, we incubated falcipain-2 with **E-64** and compound **1** in parallel; subsequently, we tested the activities of the enzyme, then dialyzed the protein bound with inhibitor in refolding buffer for 8 h and tested the activities again. In case of both inhibitors, the enzyme lost its activity after incubation (Supporting Information Figure S3A). After dialysis, no recovery of activity in the falcipain-2 incubated with **E-64** could be observed because of the covalent interaction, while the activity of the falcipain-2 incubated with compound **1** was recovered almost to the original level observed without any inhibitors owing to the noncovalent interaction (Supporting Information Figure S3B). Isothermal titration calorimetry (ITC) measurements were also performed at 25 °C; compared to **E-64**, we could not observe any significant heat changes of injection with compound **1** (data not shown). ITC measurements results showed that the interaction between compound **1** and falcipain-2 was much weaker than that between **E-64** and the protein, further verifying that our compounds bound to falcipain-2 through noncovalent interaction.

To see if there is any aggregation of falcipain-2 after incubation with our compound, we also ran gel permeation chromatography and nonreducing SDS-PAGE; the results did not indicate any aggregation (Supporting Information Figures S4A and S4B).

Conclusions

In summary, the SPECS database, consisting of approximately 287000 compounds, was screened against the falcipain-2 structure with two different docking methods. A total of 28 small molecules were identified as potential inhibitors against falcipain-2, from the 81 compounds tested, indicating a high success rate of this approach. We believe that the potential inhibitors described here may represent a starting point for finding potent molecules capable of blocking the cleavage activity of falcipain-2 toward hemoglobin. This test will form the basis for modification of the structure of the hits discovered in this work and for validation of their activity in vitro. Once a validated lead compound will be available, it can be tested in a *Plasmodium berghei* mouse model.

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Supporting Information Available: HPLC reports for the purity check of the active compounds **1**-**28**, as measured in two different mobile phases; Tanimoto similarity indices for the 28 hits; shape similarity matrix of the 28 hits; structures of compounds **9**-**28;** chemical diversity profile for 28 active compounds; enzymatic activities of refolded falcipain-2 before and after dialysis; result of gel permeation chromatography and nonreducing SDS-PAGE. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Becker, K.; Hu, Y.; Biller-Andorno, N. Infectious diseases—A global challenge. *Int. J. Med. Microbiol*. **2006**, *296* (4-5), 179–185.
(2) Vangapandu, S.; Jain, M.; Kaur, K.; Patil, P.; Patel, S. R.; Jain, R. Recent advances in antimalarial drug development. *Med. Res. Rev*. **2007**, *27* (1), 65–107.
(3) Fidock, D. A.; Rosenthal, P. J.; Croft, S. L.; Brun, R.; Nwaka, S. Antimalarial drug discovery: efficacy models for compound screening. *Nat. Rev. Drug Discovery* **2004**, *3* (6), 509–520.
(4) White, N. J. Antimalarial drug resistance. *J. Clin. Invest*. **2004**, *113* (8), 1084–1092.
(5) White, N. J. Drug resistance in malaria. *Br. Med. Bull*. **1998**, *54* (3), 703–715.
(6) Towie, N. Malaria breakthrough raises spectre of drug resistance. *Nature* **2006**, *440* (7086), 852–853.
(7) Martin, R. E.; Kirk, K. The malaria parasite's chloroquine resistance transporter is a member of the drug/metabolite transporter superfamily. *Mol. Biol. Evol*. **2004**, *21* (10), 1938–1949.
(8) Wiesner, J.; Ortmann, R.; Jomaa, H.; Schlitzer, M. New antimalarial drugs. *Angew. Chem., Int. Ed*. **2003**, *42* (43), 5274–5293.
(9) Sabnis, Y. A.; Desai, P. V.; Rosenthal, P. J.; Avery, M. A. Probing the structure of falcipain-3, a cysteine protease from *Plasmodium falciparum*: comparative protein modeling and docking studies. *Protein Sci*. **2003**, *12* (3), 501–509.
(10) Padmanaban, G. Drug targets in malaria parasites. *Adv. Biochem. Eng. Biotechnol*. **2003**, *84*, 123–141.
(11) Shenai, B. R.; Sijwali, P. S.; Singh, A.; Rosenthal, P. J. Characterization of native and recombinant falcipain-2, a principal trophozoite cysteine protease and essential hemoglobinase of *Plasmodium falciparum*. *J. Biol. Chem*. **2000**, *275* (37), 29000–29010.
(12) Dua, M.; Raphael, P.; Sijwali, P. S.; Rosenthal, P. J.; Hanspal, M. Recombinant falcipain-2 cleaves erythrocyte membrane ankyrin and protein 4.1. *Mol. Biochem. Parasitol*. **2001**, *116* (1), 95–99.
(13) Shenai, B. R.; Lee, B. J.; Alvarez-Hernandez, A.; Chong, P. Y.; Emal, C. D.; Neitz, R. J.; Roush, W. R.; Rosenthal, P. J. Structure-activity relationships for inhibition of cysteine protease activity and development of *Plasmodium falciparum* by peptidyl vinyl sulfones. *Antimicrob. Agents Chemother*. **2003**, *47* (1), 154–160.
(14) Rosenthal, P. J.; Wollish, W. S.; Palmer, J. T.; Rasnick, D. Antimalarial effects of peptide inhibitors of a *Plasmodium falciparum* cysteine proteinase. *J. Clin. Invest* **1991**, *88* (5), 1467–1472.
(15) Lee, B. J.; Singh, A.; Chiang, P.; Kemp, S. J.; Goldman, E. A.; Weinhouse, M. I.; Vlasuk, G. P.; Rosenthal, P. J. Antimalarial activities of novel synthetic cysteine protease inhibitors. *Antimicrob. Agents Chemother*. **2003**, *47* (12), 3810–3814.
(16) Desai, P. V.; Patny, A.; Sabnis, Y.; Tekwani, B.; Gut, J.; Rosenthal, P.; Srivastava, A.; Avery, M. Identification of novel parasitic cysteine protease inhibitors using virtual screening. 1. The ChemBridge database. *J. Med. Chem*. **2004**, *47* (26), 6609–6615.
(17) Desai, P. V.; Patny, A.; Gut, J.; Rosenthal, P. J.; Tekwani, B.; Srivastava, A.; Avery, M. Identification of novel parasitic cysteine protease inhibitors by use of virtual screening. 2. The available chemical directory. *J. Med. Chem*. **2006**, *49* (5), 1576–1584.
(18) Batra, S.; Sabnis, Y. A.; Rosenthal, P. J.; Avery, M. A. Structure-based approach to falcipain-2 inhibitors: synthesis and biological evaluation of 1,6,7-trisubstituted dihydroisoquinolines and isoquinolines. *Bioorg. Med. Chem*. **2003**, *11* (10), 2293–2299.
(19) Chiyanzu, I.; Hansell, E.; Gut, J.; Rosenthal, P. J.; McKerrow, J. H.; Chibale, K. Synthesis and evaluation of isatins and thiosemicarbazone derivatives against cruzain, falcipain-2 and rhodesain. *Bioorg. Med. Chem. Lett*. **2003**, *13* (20), 3527–3530.
(20) Goh, L. L.; Sim, T. S. Homology modeling and mutagenesis analyses of *Plasmodium falciparum* falcipain 2A: implications for rational drug design. *Biochem. Biophys. Res. Commun*. **2004**, *323* (2), 565–572.
(21) Sabnis, Y.; Rosenthal, P. J.; Desai, P.; Avery, M. A. Homology modeling of falcipain-2: validation, de novo ligand design and synthesis of novel inhibitors. *J. Biomol. Struct. Dyn*. **2002**, *19* (5), 765–774.
(22) Dominguez, J. N.; Leon, C.; Rodrigues, J.; Gamboa de Dominguez, N.; Gut, J.; Rosenthal, P. J. Synthesis and evaluation of new antimalarial phenylurenyl chalcone derivatives. *J. Med. Chem*. **2005**, *48* (10), 3654–3658.
(23) Greenbaum, D. C.; Mackey, Z.; Hansell, E.; Doyle, P.; Gut, J.; Caffrey, C. R.; Lehrman, J.; Rosenthal, P. J.; McKerrow, J. H.; Chibale, K. Synthesis and structure-activity relationships of parasiticidal thiosemicarbazone cysteine protease inhibitors against *Plasmodium falciparum*, *Trypanosoma brucei*, and *Trypanosoma cruzi*. *J. Med. Chem*. **2004**, *47* (12), 3212–3219.
(24) Hogg, T.; Nagarajan, K.; Herzberg, S.; Chen, L.; Shen, X.; Jiang, H.; Wecke, M.; Blohmke, C.; Hilgenfeld, R.; Schmidt, C. L. Structural and functional characterization of Falcipain-2, a hemoglobinase from the malarial parasite *Plasmodium falciparum*. *J. Biol. Chem*. **2006**, *281* (35), 25425–25437.
(25) Wang, S. X.; Pandey, K. C.; Somoza, J. R.; Sijwali, P. S.; Kortemme, T.; Brinen, L. S.; Fletterick, R. J.; Rosenthal, P. J.; McKerrow, J. H. Structural basis for unique mechanisms of folding and hemoglobin binding by a malarial protease. *Proc. Natl. Acad. Sci. U.S.A*. **2006**, *103* (31), 11503–11508.
(26) Friesner, R. A.; Banks, J. L.; Murphy, R. B.; Halgren, T. A.; Klicic, J. J.; Mainz, D. T.; Repasky, M. P.; Knoll, E. H.; Shelley, M.; Perry, J. K.; Shaw, D. E.; Francis, P.; Shenkin, P. S. Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. *J. Med. Chem*. **2004**, *47* (7), 1739–1749.
(27) Halgren, T. A.; Murphy, R. B.; Friesner, R. A.; Beard, H. S.; Frye, L. L.; Pollard, W. T.; Banks, J. L. Glide: a new approach for rapid, accurate docking and scoring. 2. Enrichment factors in database screening. *J. Med. Chem*. **2004**, *47* (7), 1750–1759.
(28) Li, H.; Li, C.; Gui, C.; Luo, X.; Chen, K.; Shen, J.; Wang, X.; Jiang, H. GAsDock: a new approach for rapid flexible docking based on an improved multipopulation genetic algorithm. *Bioorg. Med. Chem. Lett*. **2004**, *14* (18), 4671–4676.
(29) *Specs: Chemistry Solutions for Drug Discovery*, 2008; http://www.specs.net/ (accessed October 1, 2007).
(30) Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne, P. E. The Protein Data Bank. *Nucleic Acids Res*. **2000**, *28* (1), 235–242.
(31) Perola, E.; Xu, K.; Kollmeyer, T. M.; Kaufmann, S. H.; Prendergast, F. G.; Pang, Y. P. Successful virtual screening of a chemical database for farnesyltransferase inhibitor leads. *J. Med. Chem*. **2000**, *43* (3), 401–408.
(32) Rosenthal, P. J.; McKerrow, J. H.; Rasnick, D.; Leech, J. H. *Plasmodium falciparum*: inhibitors of lysosomal cysteine proteinases inhibit a trophozoite proteinase and block parasite development. *Mol. Biochem. Parasitol*. **1989**, *35* (2), 177–183.
(33) Rosenthal, P. J.; Olson, J. E.; Lee, G. K.; Palmer, J. T.; Klaus, J. L.; Rasnick, D. Antimalarial effects of vinyl sulfone cysteine proteinase inhibitors. *Antimicrob. Agents Chemother*. **1996**, *40* (7), 1600–1603.
(34) Patterson, D. E.; Cramer, R. D.; Ferguson, A. M.; Clark, R. D.; Weinberger, L. E. Neighborhood behavior: a useful concept for validation of "molecular diversity" descriptors. *J. Med. Chem*. **1996**, *39* (16), 3049–3059.
(35) *Pipeline Pilot, version 5.0*; SciTegic: San Diego, CA, 2005.
(36) Hassan, M.; Brown, R. D.; Varma-O'Brien, S.; Rogers, D. Cheminformatics analysis and learning in a data pipelining environment. *Mol. Divers*. **2006**, *10* (3), 283–299.
(37) Ballester, P. J.; Richards, W. G. Ultrafast Shape Recognition to Search Compound Databases for Similar Molecular Shapes. *J. Comput. Chem*. **2007**, *28* (10), 1711–1723.
(38) Ballester, P. J.; Richards, W. G. Ultrafast shape recognition for similarity search in molecular databases. *Proc. R. Soc. A* **2007**, *463*, 1307–1321.
(39) Zheng, S.; Luo, X.; Chen, G.; Zhu, W.; Shen, J.; Chen, K.; Jiang, H. A new rapid and effective chemistry space filter in recognizing a druglike database. *J. Chem. Inf. Model*. **2005**, *45* (4), 856–862.
(40) Bissantz, C.; Folkers, G.; Rognan, D. Protein-based virtual screening of chemical databases. 1. Evaluation of different docking/scoring combinations. *J. Med. Chem*. **2000**, *43* (25), 4759–4767.

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