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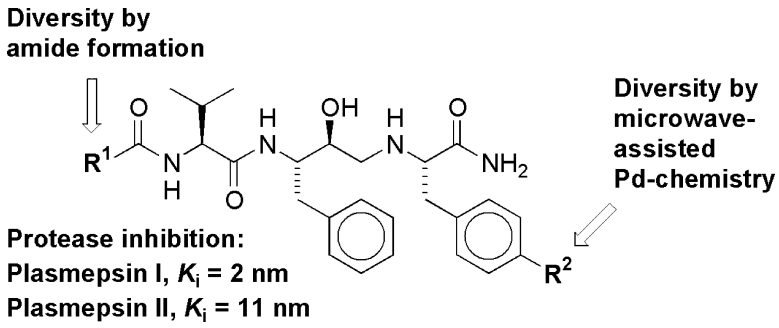

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High-Speed Optimization of Inhibitors of the Malarial Proteases Plasmepsin I and II

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Four focused libraries targeted for inhibition of the malarial proteases plasmepsin I and II were designed, synthesized, purified, and screened. Selected carboxylic acids and organometallic reactants with diverse physical properties were attached to the hydroxylethylamine scaffold in the P3 and P1′ positions to furnish inhibitors with highly improved activity. The concept of controlled and sequential microwave heating was employed for rapid library generation. This combinatorial optimization protocol afforded plasmepsin inhibitors not only with K_i values in the low nanomolar range, but also with high selectivity versus the human protease cathepsin D. With this class of inhibitory agents, modifications of the P1′ substituents resulted in the largest impact on the plasmepsin/cathepsin D selectivity.

Introduction

Malaria is endemic in many regions of the world and is second to tuberculosis as the most deadly contagious disease in the world.[1] Among the new targets studied for chemical intervention with the course of the disease, the inhibition of proteases is one of the most prominent. Approximately 2% of the genome of *Plasmodium falciparum*, the deadliest of the four subtypes of *Plasmodium* parasites, contains codes for proteases.[2,3] The two most studied aspartic proteases are plasmepsin I and II. These proteases are responsible for the initial cleavage in the hemoglobin degradation.[4,5] Thus, an efficient plasmepsin inhibition will result in the death of the malaria parasite due to starvation for amino acids. Considering this background, our principal aim is to identify potent, selective, orally bioavailable and metabolically stable plasmepsin inhibitors as potential new antimalarial drugs.[6−9]

We earlier described the synthesis and biological evaluation of a series of basic transition-state mimicking inhibitors of plasmepsin I and II with the generic structure **1** (Figure 1).[10.] The SAR of these secondary amines showed a strong preference for large R groups. In particular, the *p*-aryl-substituted benzyl derivatives (e.g., **2**) combined inhibition of the plasmepsins in the 100 nM range with a reasonable selectivity over the very similar human protease cathepsin D. Moreover, some of these compounds were found to moderately inhibit the growth of cultured *P. falciparum*-infected erythrocytes.[10] Importantly, compound **2** was also shown to likely have good oral bioavailability, as it penetrated Caco-2 cells rather well.[10]

We hereby report an attempt to optimize this class of scaffold inhibitors by rapid and convenient generation of small, targeted chemical libraries. The picolinic acid in the P3 position of the inhibitors was exchanged for a set of diverse carboxylic acids, while the other parts of the inhibitors remained constant (libraries 1 and 2). Thereafter, the P1′ benzyl group was decorated in the para position by employing palladium-catalyzed cross-coupling reactions, keeping the P3 constant as a picolinic acid amide (library 3). Finally, the side chains found to present the best inhibitors were combined to provide a fourth series (library 4) of plasmepsin inhibitors.

In medicinal and high-throughput chemistry, the overall workflow is a crucial factor, and much work is devoted to speeding up the process of chemistry development.[11−14] Since automated microwave-based synthesizers[15] are known to streamline library production and to accelerate slow organic transformations, this technology was implemented for the palladium-catalyzed coupling reactions.[16]

Results and Discussion

Library Generation. To produce targeted libraries of high diversity within the scope of the synthetic procedure, a selection of side chains to replace either the pyridine in the P3 or the terminal phenyl ring on the biphenyl function of **2** was performed. This was accomplished preceding the preparative chemistry using an experimental design technique to pick sets of reagents from larger pools of candidates (see the Experimental Section for details).

Libraries 1 and 2. The intermediate **3** was used as starting material for all the reported inhibitors in Scheme 1.[10] The Boc group was removed, and the liberated primary amine was reacted with each one of the 12 selected carboxylic acids **4**{*1*−*12*} using TBTU as a coupling agent with DIEA as base. After a quick purification, the Z group of the triamide was cleaved off using a solution of triflic acid in DCM with anisole as a scavenger. The secondary amines **5**{*1*−*10*} were thereafter isolated in 21−86% yield after this three-step

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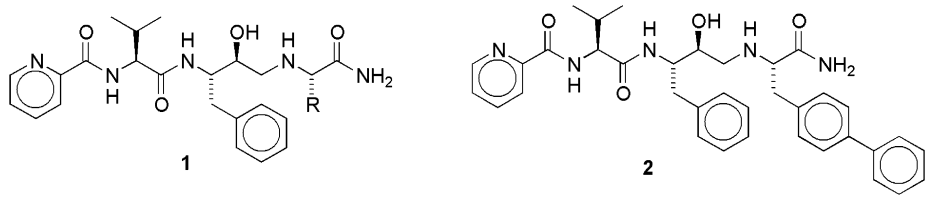
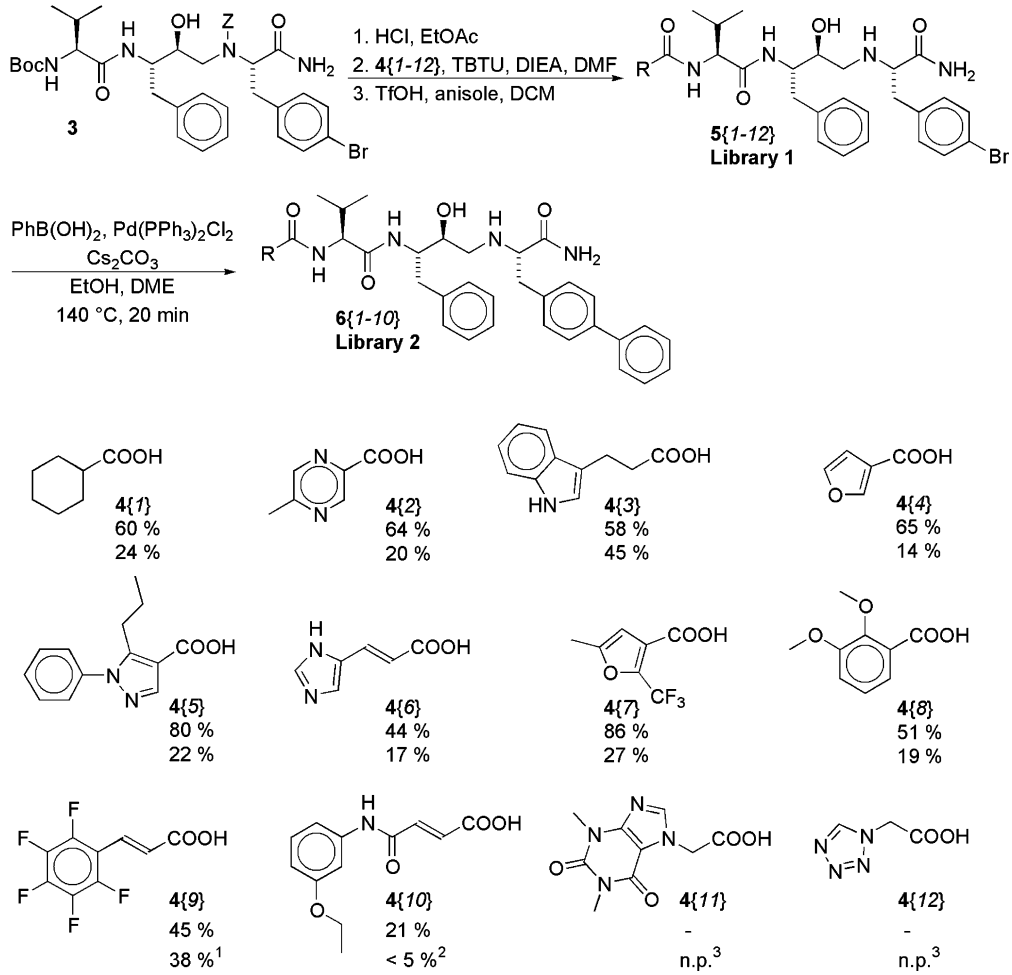


Figure 1. Generic structure from earlier investigation (**1**) and lead compound for this study (**2**).

Scheme 1. Libraries 1 and 2: Synthesis of Plasmepsin Inhibitors **5**{*1-10*} and **6**{*1-10*} with Diverse P3 Side Chains from Acids **4**{*1-10*}.[a]

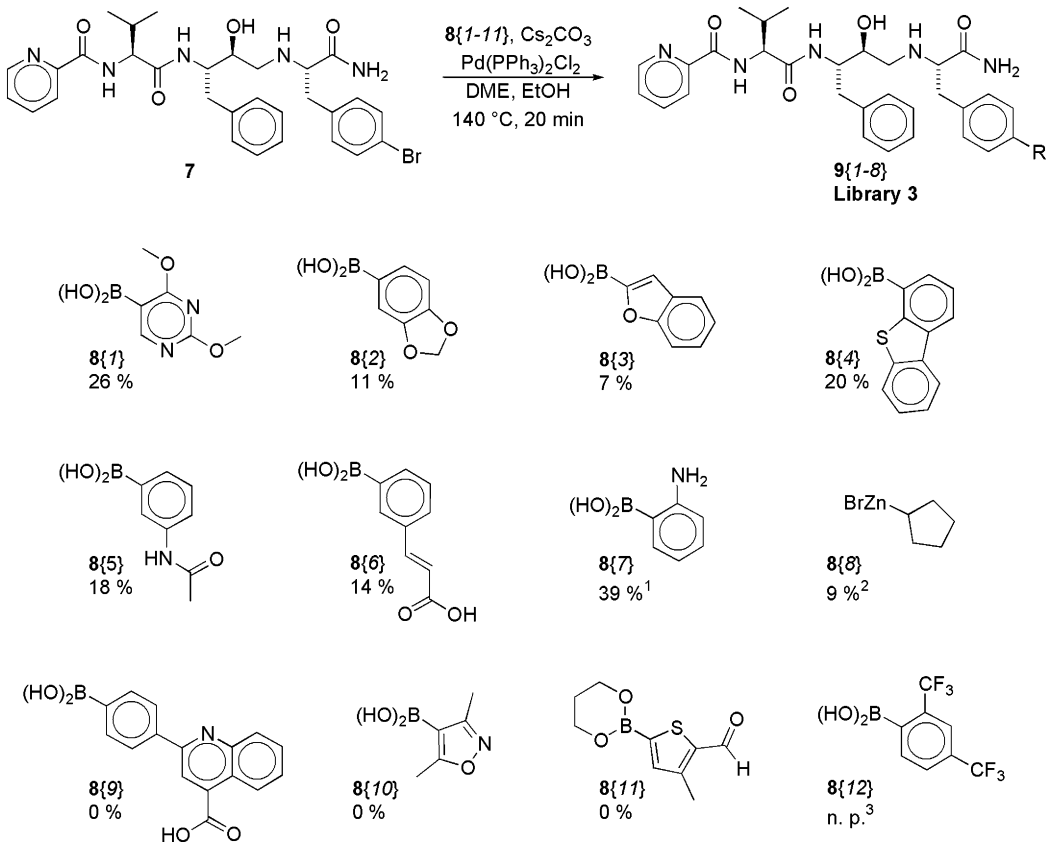


[a] Key: [1]Altered reaction conditions for the Suzuki coupling: Na_2CO_3 instead of Cs_2CO_3, 120 °C, 40 min; [2]product not isolated; [3]reaction not performed.

protocol (Scheme 1). With the electron-rich acid **4**{*10*} and anisole as the scavenger, the Z deprotection resulted in major benzylation of the activated phenyl group as deduced from the LC/MS spectrum. 1,3,5-Trimethoxybenzene was tested as an alternative scavenger, but despite this change, a considerable amount of benzylated product was formed, and the yield of **5**{*10*} did not improve (21%). Disappointingly, the isolation of **5**{*11*} and **5**{*12*} were not successful because of very low solubility in all tested solvents. The purified compounds **5**{*1*−*10*} in library 1 were subsequently subjected to rapid microwave-heated palladium-catalyzed Suzuki cross-couplings[17−20] to afford target products **6**{*1*−*10*}. Operationally, aryl bromides **5**{*1*−*10*} were reacted with 5 equiv phenylboronic acid, 8% thermostable $Pd(PPh_3)_2Cl_2$, and 3 equiv cesium carbonate in an ethanol/DME mixture (procedure A). The couplings were sequentially performed in sealed vessels under air with 20 min of controlled irradiation using an automated single-mode applicator. The phenylated products, except in the case of **6**{*10*}, were isolated in 14−45% yield after chromatography (Scheme 1). It is worth noting that no epimerization of the inhibitors was detected, despite the accelerated microwave conditions utilized. The Suzuki coupling of aryl bromide **5**{*10*} was sluggish, and starting material contaminated the product. Efforts to achieve full conversion of **5**{*10*}, for example, different temperatures and reaction times or addition of a second portion of catalyst, failed and led to a mixture that was very difficult to separate. Because the synthesis of **5**{*10*} was also problematic, an unreasonablly high amount of starting material would have had to be devoted to the synthesis of **6**{*10*}, which therefore was excluded from library 2.

Library 3. Of the selected 12 cross-coupling agents (10 boronic acids, 1 boronic ester, and 1 zink bromide) planned

Scheme 2. Library 3: Microwave Synthesis of Plasmepsin Inhibitors **9**{*1-8*} with Diverse P1′ Side Chains from Coupling Reagents **8**{*1-8*}.[a]



[a] Key: [1]Altered reaction conditions for the Suzuki coupling: Na_2CO_3 instead of Cs_2CO_3, 120 °C, 40 min. [2]Reaction conditions for Negishi coupling: $Pd(PPh_3)_2Cl_2$, dry THF, 160 °C, 20 min. [3]Reaction not performed.

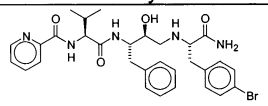
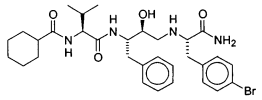
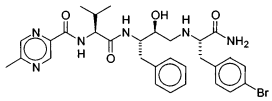
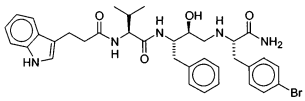
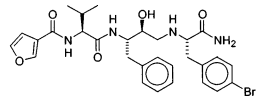
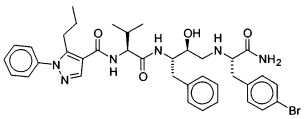
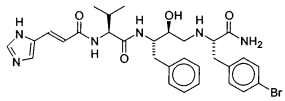
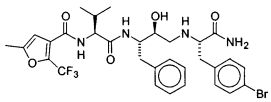
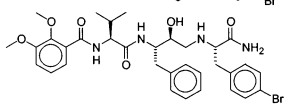
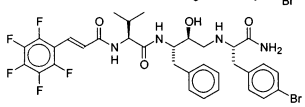
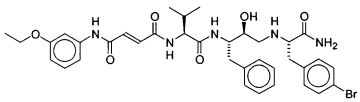
to be employed for the diverse decoration of the P1′ subunit of starting material **7** (Scheme 2), the 2,4-ditrifluoromethylphenylboronic acid **8**{*12*} was no longer commercially available. This boronic acid was thus excluded from the synthesis of library 3. The remaining 11 organometallic compounds were reacted with aryl bromide **7** under identical microwave conditions (procedure A) as described for library 2. With this protocol, eight of the coupling reactions provided enough products for characterization and biological testing (Scheme 2). However, reaction with coupling reactants **8**{*10*−*11*} furnished only dehalogenated **7**, and reactions with **8**{*7*} and **8**{*9*} provided only a complex mixture of degraded materials. Thus, prior to embarking on the synthesis of library 4, we decided to investigate new reaction conditions for the Suzuki coupling with the goal of improving the chemical yields. Screening different bases and catalytic systems at 120 °C for 40 min suggested a change from cesium carbonate to sodium carbonate. This improvement (procedure B) afforded product **9**{*7*} in 39% yield (Scheme 2) and also increased the yield of **6**{*9*} to 38% (Scheme 1).

Library 4. After biological testing of the inhibitors in libraries 1, 2, and 3, the side chains that produced the most active inhibitors were identified and combined to give a total of eight new structures (**10**{*1,3*} and **11**{*1,3*−*5,7,8*}). These compounds were synthesized using the same chemical strategy as with libraries 1−3, but employing the improved Suzuki procedure B with sodium carbonate. The structures and yields of these targeted compounds are summarized in Table 4.

Screening and Biological Evaluation. All 35 library compounds were, *first*, subjected to individual purification and chemical characterization and, *second*, evaluated for inhibition of plasmepsin I and II (Plm I and II) as well as of cathepsin D (Cat D). The results are summarized in Tables 1−4. The picolinic acid derivatives **2** and **7** have been included in the tables for comparison. The biological data disclose that the generic hydroxyethylamine scaffold functions well. All of the compounds are active on Plm I and Plm II, even though the side chains were selected to produce a large variety of physicochemical properties in the inhibitors. With very few exceptions, the library members inhibit both of the malarial enzymes in the nanomolar range. Furthermore, the inhibition always occurs with some selectivity over the human protease. Another general observation is that almost all of the compounds are more active against plasmepsin I than against plasmepsin II (except **9**{*5*} and **9**{*7*}). This is especially true for the aryl bromides **5**{*1*−*10*} produced in library 1. All of the compounds here show high selectivity for plasmepsin I, and many of the compounds are more active against the plasmepsins than the parent picolinic acid derivative **7** (Table 1). The most marked Plm/Cat D selectivity is found in the indole derivative **5**{*3*}, with an ∼20 times improvement against plasmepsin I and ∼4 times lower K_i against plasmepsin II, as compared to the parent compound **7**.

With the different aryl bromide derivatives, a further *p*-phenyl extension in the P1′ in all cases resulted in an improved binding to both Plm I and Plm II (compare Tables

Table 1. Biological Evaluation of Library 1

Library 1	Name	K_i (Plm I / nM)	K_i (Plm II / nM)	K_i (Cat D / nM)
	**7**	98	540	>2000
	**5**{*1*}	29	490	>2900
	**5**{*2*}	240	1100	>2900
	**5**{*3*}	4.7	140	500
	**5**{*4*}	200	1200	>2900
	**5**{*5*}	46	260	920
	**5**{*6*}	340	1100	>2900
	**5**{*7*}	130	510	640
	**5**{*8*}	180	810	>2900
	**5**{*9*}	27	660	3400
	**5**{*10*}	63	690	>2900

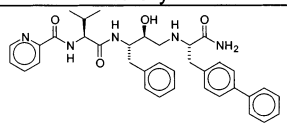
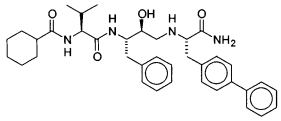
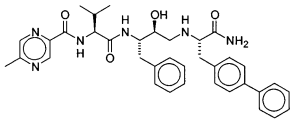
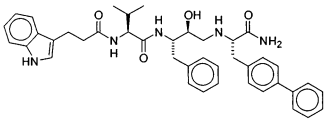
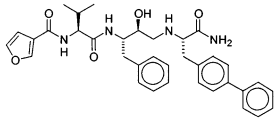
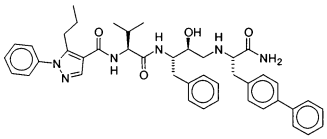
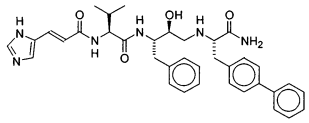
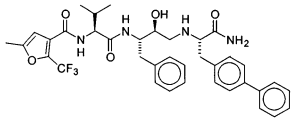
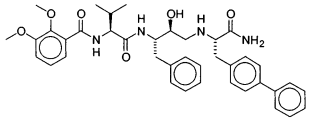
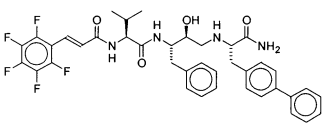
1 and 2). Again, an indole-derived compound, **6**{*3*}, is the library 2 member that stands out as the most active inhibitor of the series, with a 10-fold reduction in the K_i value for Plm II after phenylation.

The fact that the side chains were selected to be diverse makes it difficult to find a clear structure−activity relationship for the P3 substitution in libraries 1 and 2. Size apparently does not matter very much; neither does polarity seem to play an easily interpretable part in binding the S3 subsite. However, one conclusion is apparent. The inhibition results indicate that the S3 of Cat D is very similar to the S3 of the plasmepsins. Thus, the compounds that are more active against the plasmepsins also tend to be more active against Cat D, although there is always some degree of selectivity for the malaria proteases, especially with highly active **6**{*1*} (Plm I and Plm II vs. Cat D).

With respect to the substitution pattern of the P1′ side chain in library 3, we had earlier realized that an *o*-methoxyphenyl substitution on the para position of the P1′ benzyl ring of **3** lowered biological activity considerably.[10] Therefore, we were not surprised to encounter moderate activity with compounds **9**{*1*}, **9**{*4*}, and **9**{*7*}, although the activity of **9**{*4*} was even lower than expected. Nor does a meta substituent on the terminal P1′ phenyl ring strongly enhance the potency (**9**{*5*} and **9**{*6*} vs biphenyl **2**), whereas larger bicyclic and oxygen-containing ring systems in the space of meta and para substituents do, indeed, appear to be favorable (**9**{*2*} and **9**{*3*}). The small cyclopentane ring renders **9**{*8*} even less active than the corresponding aryl bromide, perhaps because of the different "out of the plane" geometry, as compared with the aromatic substituents. The S1′ pocket of Cat D probably has much less in common with the plasmepsins than the S3 subsite of Cat D. For example, the two most plasmepsin-active compounds in library 3, **9**{*2*} and **9**{*3*}, are much less potent against Cat D, whereas the cinnamic acid derivative **9**{*6*} gains much more inhibitory power against Cat D than it does for the plasmepsins.

Library 4 was designed by combining some of the best P3 and P1′ side chains detected in libraries 1−3. In this set of eight new compounds, none proved to have greater activity against the plasmepsins than those that had already been established in previous libraries, although **10**{*3*} and **11**{*3*}

Table 2. Biological Evaluation of Library 2

Library 2	Name	K_i (Plm I / nM)	K_i (Plm II / nM)	K_i (Cat D / nM)
	**2**	68	120	>2000
	**6**{*1*}	12	43	1900
	**6**{*2*}	82	190	>2900
	**6**{*3*}	2.6	11	30
	**6**{*4*}	18	57	2200
	**6**{*5*}	33	62	1700
	**6**{*6*}	150	150	>2900
	**6**{*7*}	55	70	3200
	**6**{*8*}	24	110	570
	**6**{*9*}	2	120	1400

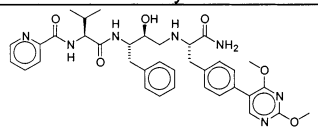
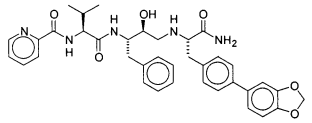
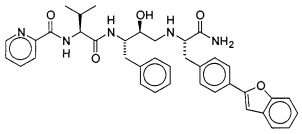
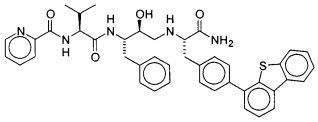
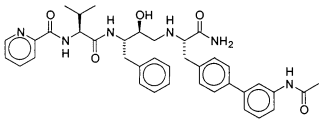
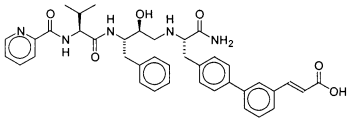
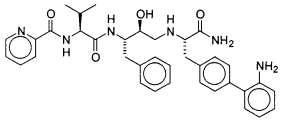
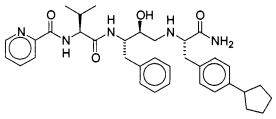
were active in the single-digit nanomolar range for Plm I (Table 4). Thus, it appears that one has to be very careful to balance the P1′ and P3 side chains to obtain maximum potency. It is noteworthy that the 3-(3-indol)-propanoic acid acts as a very general P3 side chain in the investigated structures (**5**{*3*}, **6**{*3*}, **10**{*3*}, and **11**{*3*}. There is, however, one P3 group included in the series that consistently display good selectivity. The cyclohexyl amide in compounds **6**{*1*}, **11**{*1*}, and most markedly, **10**{*1*} gives in all cases fairly active and very selective plasmepsin inhibitors.

Conclusions

Reported here are the results of a search for improved antimalarial plasmepsin I and II inhibitors. The rapid and efficient generation of targeted hydroxyethylamine based libraries with different P3 and P1′ substituents were envisioned to be of importance for the lead development process. Compared to conventional thermal heating, single-mode microwave irradiation accelerated the library synthesis step by reducing the reaction times of the Suzuki reactions from h to min.

With regard to Plm I and II inhibition, it was demonstrated that very diverse side chains are tolerated in both the P3 and P1′ positions of the hydroxyethylamine transition state isostere. Furthermore, a number of synthesized compounds were identified that combined high malarial protease inhibition (in the low nanomolar range) with high selectivity against the similar human aspartic protease cathepsin D. From the information presented herein, the focus for future investigations of the generic hydroxyethylamine scaffold should be on the P1′ end of the inhibitor, since the plasmepsin/cathepsin selectivity was found to be partly

Table 3. Biological Evaluation of Library 3

Library 3	Name	K_i (Plm I / nM)	K_i (Plm II / nM)	K_i (Cat D / nM)
	**9**{*1*}	170	360	>2900
	**9**{*2*}	23	52	1900
	**9**{*3*}	13	30	1400
	**9**{*4*}	220	490	>2900
	**9**{*5*}	99	92	>2900
	**9**{*6*}	42	110	300
	**9**{*7*}	610	600	>5900
	**9**{*8*}	130	1300	>2900

controlled in this area. We suggest that a possible combined P3/P1′ strategy for further chemistry efforts might involve the investigation of nonaromatic P3 side chains. Finally, to achieve good pharmacokinetic properties, the molecular weight will need to be reduced.

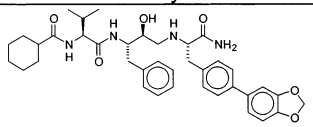
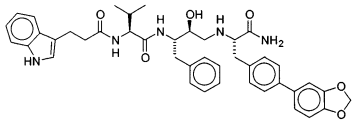
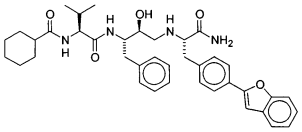
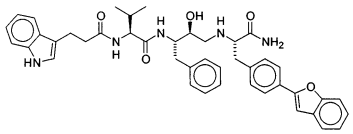
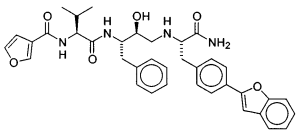
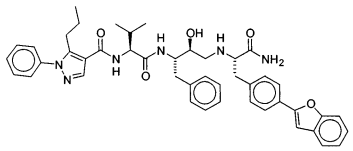
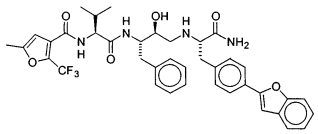
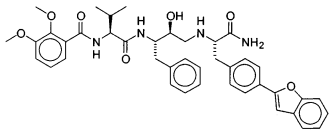
Experimental Section

Selection of Side Chain Precursors. Chemical descriptors were calculated for the carboxylic acid and boronic acid candidate sets in order to allow the selection of a diverse set of side chains. 2D fingerprints, calculated with Unity software,[21] were used as the metric, since they are both easy to calculate and appropriate for bioactive selections.[22] The fingerprints were expressed as a 992-bit string encoding information related to the chemical connectivity of each candidate. Differences between pairs of candidates were determined by calculation of Tanimoto coefficients,[23] which express the percentage of similarity by a comparison of fingerprints. The selection of side chains was accomplished using an experimental design technique based on the method of "maximum-dissimilarity".[24] This procedure was used to initially select into the design set the pair of candidates that were determined to be most different by some metric of the candidate set. For each remaining candidate, the distance between the candidate and the closest (i.e., most similar) member of the design set is tallied. The candidate that has the greatest of these minimum distances (i.e., the one which is furthest away from those already chosen) is added to the design set. Additional candidates are selected in the same manner, maximizing the minimum distance to the compounds already in the design set.

As implemented here, alternative starting points for the algorithm are possible. Some compounds could be preselected to satisfy an important bias or to incorporate compounds of a previously synthesized library. This technique was utilized in the selection of the second half of the carboxylic acid set (vide infra). For a small pool of candidates, this preselection procedure could be expanded to search for more favorable datasets by iteratively running through the calculation starting from each candidate in turn. This variation was used in the selection of boronic acids (vide infra).

Carboxylic Acids. From a collection of carboxylic acids readily available in our laboratory, 69 were considered suitable on the basis of criteria such as having a molecular weight below 300 and the absence of undesired functional groups (especially those which were expected to interfere with the subsequent reactions). For example, vinylic, allylic,

Table 4. Synthesis and Biological Evaluation of Library 4

Library 4	Name	Yield	K_i (Plm I / nM)	K_i (Plm II / nM)	K_i (Cat D / nM)
	**10**{*1*}	64 %	12	110	3300
	**10**{*3*}	38 %	4.8	65	280
	**11**{*1*}	72 %	35	43	1200
	**11**{*3*}	62 %	4.1	95	180
	**11**{*4*}	12 %	54	110	1100
	**11**{*5*}	46 %	54	140	570
	**11**{*7*}	75 %	160	160	1700
	**11**{*8*}	77 %	20	85	1000

acidic, and aromatic halides were eliminated. Six of these carboxylic acids, **4**{*1,2,4,6,8,9*}, were chosen for the library using the reagent selection method described above.

The ACD[25] was searched for additional carboxylic acids. The same criteria for the locally available acids were used to refine the search from the complete collection of ~40 000 commercially available acids to ~4000 candidates. Taking the six previously chosen acids into consideration, an additional six, **4**{*3,5,7,10*−*12*}, were selected from this pool of candidates using the same reagent selection method to increase the diversity of the library.

Cross-Coupling Reactants. A total of 224 commercially available boronic acids and boronic esters were selected from the ACX[26] database of available chemicals. Using filters similar to those used for the carboxylic acids, candidates possessing unwanted functional groups were eliminated from consideration. For example, boronic acids or esters containing Cl, Br, or I were removed from the list since they were expected to interfere with the coupling reaction. For purposes of estimating diversity, the boronic esters were considered equivalent to the corresponding boronic acids. The candidate set consisted of the remaining 152 unique compounds. Twelve structurally diverse boronic acids and esters, **8**{*1*−*7,9*−*12*}, were chosen using the reagent selection method described above. The zinc bromide **8**{*8*} was a replacement for the equivalent boronic acid from this set.

Plasmepsin Assay and K_i Determination. Pro-plasmepsin II was a generous gift from Helena Danielson (Department of Biochemistry, Uppsala University, Uppsala, Sweden), and the expression and purification of plasmepsin I will be published elsewhere (manuscript in preparation).

Human liver cathepsin D was purchased from Sigma-Aldrich, Sweden. The activities of plasmepsin I (Plm I), plasmepsin II (Plm II), and cathepsin D were measured essentially as described earlier[9] using a total reaction volume of 100 μL. The concentration of pro-Plm II was 3 nM, the

amount of Plm I was adjusted to give similar catalytic activity, and 50 ng/mL pro-cathepsin D was used. The pro sequence of Plm II was cleaved off by preincubation in the assay reaction buffer (100 mM sodium acetate buffer [pH 4.5]), 10% glycerol, and 0.01% Tween 20) at room temperature for 40 min, and cathepsin D was activated by incubation in the same reaction buffer at 37 °C for 20 min.

The reaction was initiated by the addition of 3 μM substrate DABCYL-Glu-Arg-Nle-Phe-Leu-Ser-Phe-Pro-EDANS (AnaSpec Inc, San Jose, CA), and hydrolysis was recorded as the increase in fluorescence intensity over a 10-min interval, during which the rate increased in a linear fashion.

Stock solutions of inhibitors in DMSO were serially diluted in DMSO and added directly before addition of substrate, giving a final DMSO concentration of 1%.

IC_{50} values were obtained by assuming competitive inhibition and fitting a Langmuir isotherm ($v_i/v_o = 1/(1 + [I]/IC_{50})$) to the dose response data (Grafit), where v_i and v_o are the initial velocities for the inhibited and uninhibited reaction respectively and [I] is the inhibitor concentration.[27] The K_i was subsequently calculated by using $K_i = IC_{50}/(1 + [S]/K_m)$[28], and a K_m value was determined according to Michaelis−Menten.

Chemistry. General. All microwave reactions were conducted in heavy-walled glass Smith process vials sealed with aluminum crimp caps fitted with a silicon septum. The microwave heating was performed in a Smith Synthesizer single-mode microwave cavity producing continuous irradiation at 2450 MHz (Personal Chemistry AB, Uppsala, Sweden). Reaction mixtures were stirred with a magnetic stirring bar during the irradiation. The temperature, pressure, and irradiation power were monitored during the course of the reaction. After completed irradiation, the reaction tube was cooled with high-pressure air until the temperature had fallen below 39 °C. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-EX 270 spectrometer at 270.2 and 67.8 MHz, respectively, or on a JEOL JNM-EX 400 spectrometer at 399.8 and 100.5 MHz, respectively. Flash column chromatography was performed on Merck silica gel 60, 0.04−0.063 mm. Thin-layer chromatography was performed using aluminum sheets precoated with silica gel 60 F_{254} (0.2 mm; E. Merck) and visualized with UV light and ninhydrin. Analytical RP-LC/MS was performed on a Gilson HPLC system with a Zorbax SB-C8, 5 μm 4.6 × 50 mm (Agilent Technologies) column, with a Finnigan AQA quadropole mass spectrometer at a flow rate of 1.5 mL/min (H_2O/CH_3CN/0.05% HCOOH).

General Procedure for the Synthesis of Compounds 5{*1*−*10*}. (2*S*,5*S*,6*R*)-3-Aza-3-(benzyloxycarbonyl)-2-(*p*-bromobenzyl)-6-{[(*tert*-butyloxycarbonyl)-L-valinyl]amino}-5-hydroxy-7-phenylheptanoyl amide (**3**, ∼410 mg, 0.66 mmol) was dissolved in a mixture of DCM and TFA (1:1, 15 mL), and the resulting solution was stirred at room temperature for 15 min. The solvents were removed by evaporation. The residue was suspended in aqueous Na_2CO_3 (15 mL, 2 M) and extracted three times with DCM (15 mL). The combined organic phases were dried, filtered, and evaporated. The resulting primary amine was used without further purification and dissolved in DMF (2 mL). The carboxylic acid (**4**{*1*−*10*}, 1.1 equiv), TBTU (1.1 equiv), and DIEA (2.2 equiv) were added, and the mixture was stirred at room temperature for 2 h. DCM (20 mL) was added, and the mixture was washed with aqueous $NaHCO_3$ (20 mL, 1 M). The organic phase was dried, filtered, and evaporated, and the residue was purified by filteration through a short silica column (mobile phase DCM/MeOH 99:1 → 19:1) to give the corresponding amide. This amide was dissolved in DCM (100 mL), anisole (2 equiv) was added, and the mixture was stirred for 5 min. Triflic acid (10 equiv) was added, and the mixture was stirred vigorously for an additional 20 min. Aqueous Na_2CO_3 (sat.) was added until the pH was >10, water (30 mL) was added, and the phases were separated. The organic phase was dried, filtered, and evaporated. The residue was purified by column chromatography (mobile phase DCM/MeOH 39:1 → 9:1) to give the corresponding secondary amines **5**{*1*−*10*}.

5{*1*}. Yield: 60%. ^{1}H NMR (270 MHz, CD_3OD) δ 0.74−0.86 (m, 6 H), 1.10−1.48 (m, 5 H), 1.59−1.71 (m, 1 H), 1.71−1.82 (m, 4 H), 1.82−1.99 (m, 1 H), 2.03−2.19 (m, 1 H), 2.38−2.47 (m, 2 H), 2.66−2.87 (m, 3 H), 2.93 (dd, J = 5.6, 13.9 Hz, 1 H), 3.20 (dd, J = 5.5, 8.0 Hz, 1 H), 3.47−3.55 (m, 1 H), 3.99−4.09 (m, 2 H), 7.02−7.10 (m, 2 H), 7.10−7.27 (m, 5 H), 7.34−7.43 (m, 2 H). ^{13}C NMR (67.9 MHz, CD_3OD) δ 17.6, 18.9, 25.4, 29.0, 29.5, 30.4, 37.6, 38.4, 44.9, 51.0, 52.4, 58.3, 63.2, 69.5, 120.3, 120.0, 128.0, 128.9, 130.6, 131.3, 136.0, 137.7, 171.5, 176.9, 177.0.

General Procedure A for the Suzuki Couplings. A 0.5-mL Smith vial was charged with the aryl bromide (∼20−30 mg), the boronic acids (5 equiv), Cs_2CO_3 (3 equiv), $Pd(PPh_3)_2Cl_2$ (0.08 equiv), DME (0.4 mL), and ethanol (0.1 mL). The vial was irradiated in a Smith synthesizer at 140 °C for 20 min. The contents of the vial were filtered through a Celite plug, which was washed out thoroughly with methanol. The filtrate was evaporated, and the residue was purified by column chromatography (mobile phase DCM/MeOH 39:1 → 9:1) to give the arylated product.

General Procedure B for the Suzuki Couplings. Same as procedure A, with Na_2CO_3 instead of Cs_2CO_3 and irradiation at 120 °C for 40 min.

6{*1*}. Procedure A, 24% yield. ^{1}H NMR (400 MHz, $CDCl_3$, CD_3OD 3:1) δ 0.57−0.66 (m, 6 H), 0.96−1.27 (m, 5 H), 1.44−1.53 (m, 1 H), 1.54−1.65 (m, 4 H), 1.65−1.79 (m, 1 H), 1.90−2.00 (m, 1 H), 2.35−2.48 (m, 2 H), 2.61 (dd, J = 8.5, 13.8 Hz, 1 H), 2.70 (dd, J = 7.0, 13.8 Hz, 1 H), 2.77 (dd, J = 6.6, 13.7 Hz, 1 H), 2.91 (dd, J = 6.3, 13.4 Hz, 1 H), 3.25−3.42 (m, 1 H), 3.42−3.56 (m, 1 H), 3.78−3.88 (m, 2 H), 6.90−7.08 (m, 4 H), 7.08−7.19 (m, 3 H), 7.19−7.28 (m, 3 H), 7.31−7.41 (m, 4 H).

9{*1*}. Procedure A, 26% yield. ^{1}H NMR (400 MHz, $CDCl_3$, CD_3OD 3:1) δ 0.67 (d, J = 6.9 Hz, 3 H), 0.71 (d, J = 6.9 Hz, 3 H), 1.88−2.00 (m, 1 H), 2.63−2.95 (m, 5 H), 3.04 (dd, J = 9.4, 13.6 Hz, 1 H), 3.22−3.28 (m, 1 H), 3.86−3.94 (m, 2 H), 3.87 (s, 1 H), 3.90 (s, 3 H), 4.04−4.09 (m, 1 H), 6.77−6.88 (m, 1 H), 6.91−7.11 (m, 4 H), 7.17−7.48 (m, 5 H), 7.72−7.81 (m, 1 H), 7.94−8.01 (m, 1 H), 8.04−8.10 (m, 1 H), 8.44−8.49 (m, 1 H).

11{*1*}. Procedure B, 72% yield. ^{1}H NMR (400 MHz, $CDCl_3$, CD_3OD 3:1) δ 0.53−0.66 (m, 6 H), 0.91−1.28 (m,

6 H), 1.41−1.52 (m, 1 H), 1.52−1.65 (m, 4 H), 1.65−1.77 (m, 1 H), 2.41−2.78 (m, 4 H), 2.82−3.00 (m, 2 H), 3.13−3.21 (m, 1 H), 3.49−3.59 (m, 1 H), 3.59−3.67 (m, 1 H), 3.75−3.83 (m, 1 H), 6.78−6.86 (m, 1 H), 6.90−7.10 (m, 8 H), 7.11−7.19 (m, 1 H), 7.25−7.33 (m, 1 H), 7.33−7.44 (m, 1 H), 7.58−7.66 (m, 1 H).

9{*8*}. A 0.5-mL Smith vial was charged with (2*S*,5*S*,6*R*)-3-aza-2-(*p*-bromobenzyl)-5-hydroxy-7-phenyl-6-[(picolyl-L-valinyl)amino]-heptanoyl amide (**7**, 34 mg, 56 μmol), $Pd(PPh_3)_2Cl_2$ (3 mg, 4.3 μmol), and cyclopentyl zink bromide (0.5 M in THF, 1 mL, 0.5 mmol) and irradiated in a Smith synthesizer for 20 min at 160 °C.[29] The contents of the vial were filtered through a Celite plug that was washed out with methanol. The filtrate was evaporated, and the residue was purified by column chromatography (mobile phase DCM/MeOH 39:1 → 9:1) to give the product **9**{*8*} (3 mg, 9%) as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$, CD_3OD 3:1) δ 0.66 (d, J = 6.8 Hz, 3 H), 0.77 (d, J = 6.8 Hz, 3 H), 1.37−1.52 (m, 2 H), 1.53−1.65 (m, 2 H), 1.65−1.77 (m, 2 H), 1.89−2.06 (m, 3 H), 2.73−2.96 (m, 6 H), 3.03 (dd, J = 9.5, 12.3 Hz, 1 H), 3.24 (dd, J = 4.8, 13.6 Hz, 1 H), 3.99−4.19 (m, 3 H), 6.91−6.99 (m, 1 H), 7.03−7.18 (m, 8 H), 7.59−7.66 (m, 1 H), 8.02−8.10 (m, 1 H), 8.25−8.32 (m, 1 H), 8.60−8.67 (m, 1 H).

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Supporting Information Available. Isolated yields and NMR data of compounds **5**{*2*−*10*}, **6**{*2*−*9*}, **9**{*2*−*7*}, **10**{*1*,*3*}, and **11**{*3*−*5*,*7*,*8*}. This material is available free of charge via the Internet at http://pubs.acs.org.

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