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DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF PEPTIDOMIMETIC N-SUBSTITUTED CBZ-4-HYP-HPA-AMIDES AS NOVEL INHIBITORS OF *PLASMODIUM FALCIPARUM*

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A new series of peptidomimetic N-substituted Cbz-4-Hyp-Hpa-amides was designed, synthesized and evaluated for inhibition of the *Plasmodium falciparum*. Substituents on the N atom of the amide group were selected. They are the following alkyl-, allyl-, aryl-, 2-hydroxyethyl-, 2-cyanoethyl-, cyanomethyl-, 2-hydroxyethyl-, 2,2-diethoxyethyl-, or 2-ethoxy-2-oxoethylamino groups, and about forty new compounds were synthesized and evaluated for anti-plasmodial activity *in vitro*. Antimalarial activity was investigated as for the final peptide mimetics, and their immediate predecessors, carrying TBDMS or TBDPS protecting groups on 4-hydroxyproline residue and eighteen derivatives exhibited toxicity against *P. falciparum*. Of these agents, compound **23e** was shown to have potent antimalarial activity with IC_{50} 528 ng/mL.

Key words: antimalarial activity, peptidomimetic, falcipain, drug design, synthesis.

1. Introduction

Malaria, trypanosomiasis, and leishmaniasis are major parasitic diseases in many countries. Recent WHO data shows approximately 429,000 deaths from malaria, mainly due to the influence of the parasite *Plasmodium falciparum* [1]. The existing chemotherapy of these diseases suffers from the evolution of widespread drug resistance and/or lack of safe and effective drugs.

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The search and use of new drugs directed against malarial proteases is required among various methods of malaria treatment, combating parasitic infections by chemotherapy approach [2]. Plasmodium falcipains and other cysteine proteases are involved in various processes of the erythrocytic cycle of the malaria parasite and are exciting targets for antiparasitic drug design [3]. Although the key catalytic role of *Plasmodium falciparum* enzymes, principal food vacuolar hemoglobinases, falcipain-2 (FP-2) and falcipain-3 (FP-3) is generally known, the degradation of hemoglobin, as source of amino acids for parasite development, is carried out by enzymatic complex which contains several parasite proteins, also including plasmepsin II, plasmepsin IV, histo aspartic protease, and heme detoxification protein (HDP) [4-6].

The majority of previous studies was devoted to the inhibitors search of FP-2 and FP-3 [4] and HDP was only recently proposed as a drug target [7]. Of the two groups of mechanism-based cysteine protease inhibitors, preference should be given to reversible antiparasitic agents, which are considered potentially more effective than irreversible ones [8, 9].

The results of synthetic [10-15], protein modelling, docking studies [16] and virtual screening studies [9, 15, 17-20], led to the conclusion that in order to obtain highly active agents, efforts should be made to the design of peptidomimetic molecules with high affinity to the binding site of the enzyme.

P. J. Rosenthal et. al. investigated several peptide fluoromethyl ketone proteinase inhibitors, constituted as combination Phe-Arg, Phe-Ala, Phe-Phe or Tyr-Ala derivatives, from which benzyl (S)-1-((R)-1-fluoro-6-guanidino-2-oxohexan-3-ylamino)-1-oxo-3-phenylpropan-2-ylcarbamate (**1**, (Z)-Phe-Arg-CH_2F, Fig. 1) was the most potent inhibitor. Peptidomimetic compound **1** inhibited *P. falciparum* trophozoite cysteine proteinase (TCP), blocked hemoglobin degradation and killed parasites at picomolar concentrations [10]. Further research within this group of compounds revealed such highly potent inhibitors as Mu-Phe-HPhe-CH_2F (**2**) [11], Mu-Leu-HphVSPh (**3**) [12] and *N*-Me-pipu-Leu-Hph-VS2Np (**4**) [13].

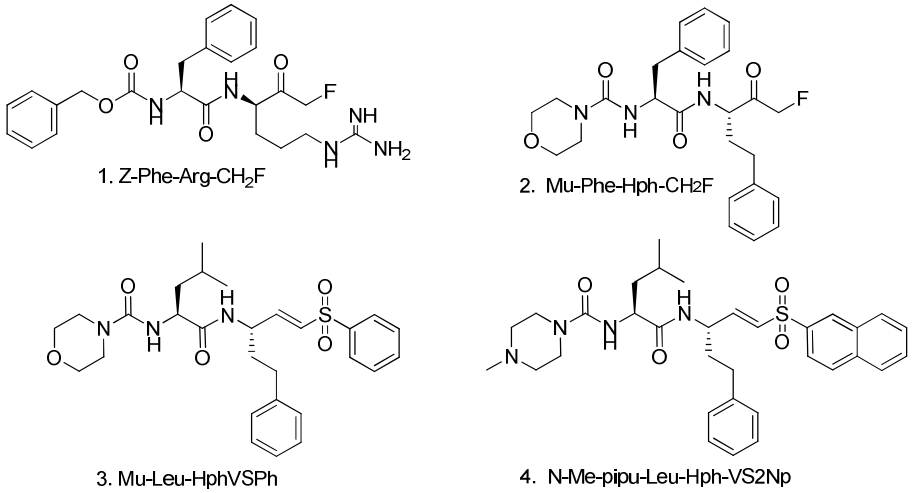
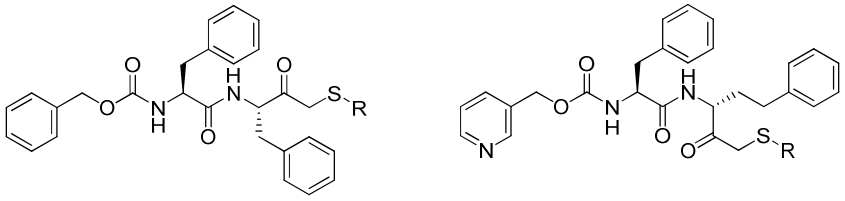


Fig. 1. Early structures of the peptidyl cysteine protease inhibitors

Peptidomimetic vinyl sulfones like **3** and **4** (Fig. 1), playing the role of Michael acceptors, tend to form covalent bond with the thiolate of the catalytic cysteine within active site that make them irreversible inhibitors [21, 22]. However, cell permeability and the selectivity of vinyl sulfones towards parasitic protease in comparison with human cysteine protease should be definitely improved [22]. Similar results were also shown in the study of irreversible inhibitors containing fumaric acid as an electrophilic building block, which can covalently link the active site Cys residue of FP-2 and rhodesain via a Michael-type reaction [23], [24].

Several ketone-based peptides (**5**, **6**, Fig. 2) were reported to be potent and reversible inhibitors of cruzain, cysteine protease implicated in Chagas' disease [25].

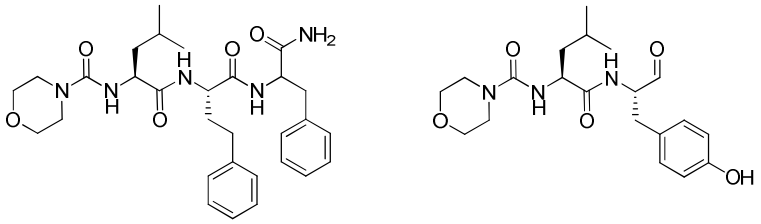


5. Cbz-Phe-Phe Mercaptomethyl Ketones

6. Py-Phe-hPhe Mercaptomethyl Ketones

Fig. 2. Peptidyl ketone-based cysteine protease (cruzain) inhibitors

A number of di- and tri-peptides was investigated, some of which (**7** and **8**, Fig. 3) exhibited antiparasitic activity in the picomolar range [26].

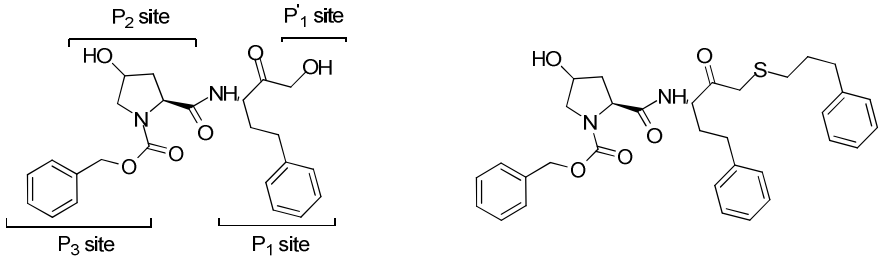


7. Morpholino-CO-Leu-hPhe-(CO)-Phe-NH_2

8. Morpholino-CO-Leu-Tyr-al

Fig. 3. Structures of the reversible peptidomimetic malaria parasite inhibitors

Recently, series of peptidomimetic hydroxyprolyl-homophenylalanylketones were designed, synthesized and evaluated for inhibition of the *Plasmodium falciparum* cysteine proteases FP-2 and FP-3 [15]. Analysis of structures mostly potent peptidomimetics **9** (IC_{50} 80 nM against FP-2 and 60 nM against FP-3), **10** (IC_{50} 1.10 µM against FP-2 and 0.52 µM against FP-3) (Fig. 4) and other derivatives from this series in comparison with structural motif of potent inhibitors of cysteine proteases was evident that hydroxyproline in the P_2 pocket (see Fig. 4), an α-hydroxyketone electrophile in the P'_1 pocket, and homophenylalanine in the P_1 pocket were suitable substituents for high binding affinity of these peptidomimetics.



9. Cbz-4-HYP-HPA-CH_2OH

10. Cbz-4-HYP-HPA-$CH_2S(CH_2)_3Ph$

Fig. 4. Structures of the peptidomimetic hydroxyprolyl-homophenylalanylketones

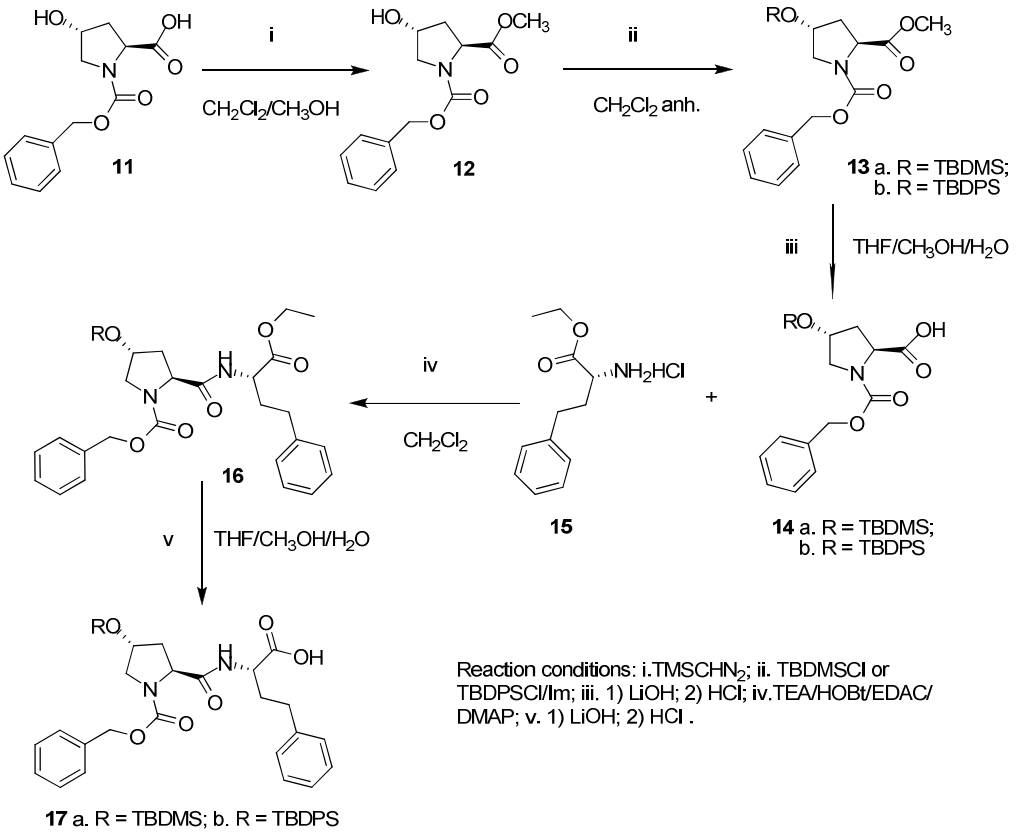
The authors proposed to formulate the residues of unnatural amino acids into anti-malarial peptidomimetic inhibitors, for example, L-homophenylalanine fragment in this series of derivatives. It was assumed that these peptides would be more resistant to peptidase and would have an increased half-life. Furthermore, the design can lead to decreased analogue flexibility and improved binding thermodynamics as well as introduction of rigid L-proline residue [15].

It can be concluded from the data of the antimalarial activity of the discussed compounds, that their design was quite productive and the substances **9** and **10** can be accepted as the lead compounds in this group of peptidomimetics.

2. Results and discussion

2.1. Chemistry

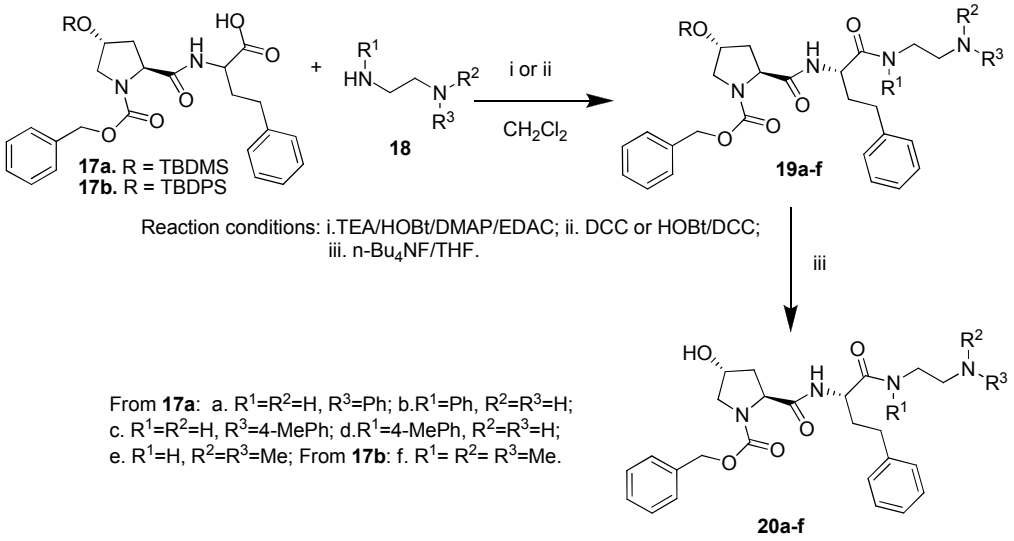
It was previously found that high activity was observed for compounds composed of 4-hydroxy-L-proline and L-homophenylalanine constituents [15]. Based on these data, corresponding dipeptide **17**, consisting of protected 4-hydroxy-L-proline and L-homophenylalanine residues with free C-terminus available for modification was chosen as a key intermediate. The major variances of structure made to these analogues were substitution of the C-terminus of L-homophenylalanine moiety related to area of P'1 site of our model.



Scheme 1. Synthesis of key dipeptide **17**

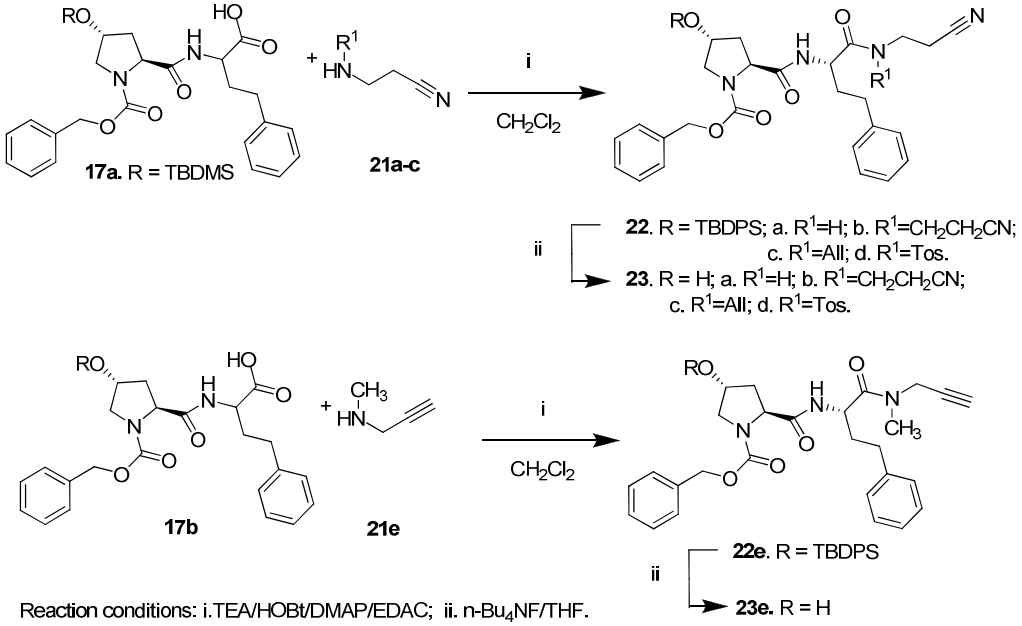
Intermediate dipeptide **17** was synthesized in five steps (Scheme 1) starting from N-Cbz-4-hydroxy-L-proline (**11**) by sequential introduction of tert-butyldimethyl (TBDMS) or tert-butyldiphenyl (TBDPS) silyl protective groups, which were mostly appropriate for the following transformations [27]. Obtained silyl protected N-Cbz-4-hydroxy-L-proline (**14**) then was condensed with ethyl L-homophenylalanine HCl (**15**) and saponification of **16** provided with desired dipeptide **17**. Substances for antimalarial testing were obtained by condensing C-terminus of **17** with substituted amines.

According to the chosen strategy obtained dipeptide **17** was reacted with substituted ethylenediamines **18** to form intermediates **19** and then TBDMS (or TBDPS) protecting group was removed by standard procedure [27] to give desired peptidomimetics **20** (Scheme 2).



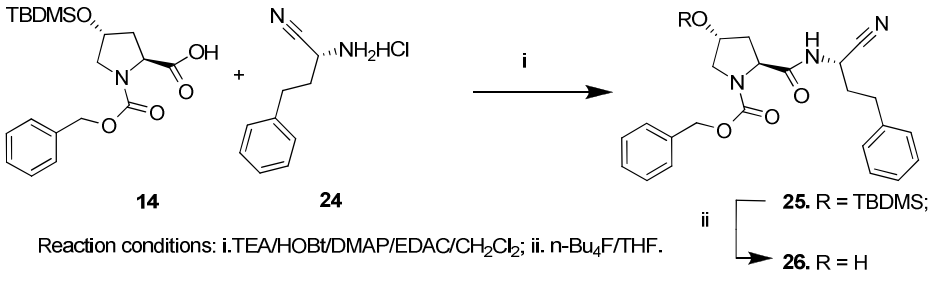
Scheme 2. Synthesis of peptidomimetic inhibitors **20**

We also synthesized a number of derivatives **22** and **23a** bearing amino ethylencyano-substituents at P'_1 site (Scheme 3).



Scheme 3. Synthesis of cyano-substituted peptidomimetic inhibitors **23**

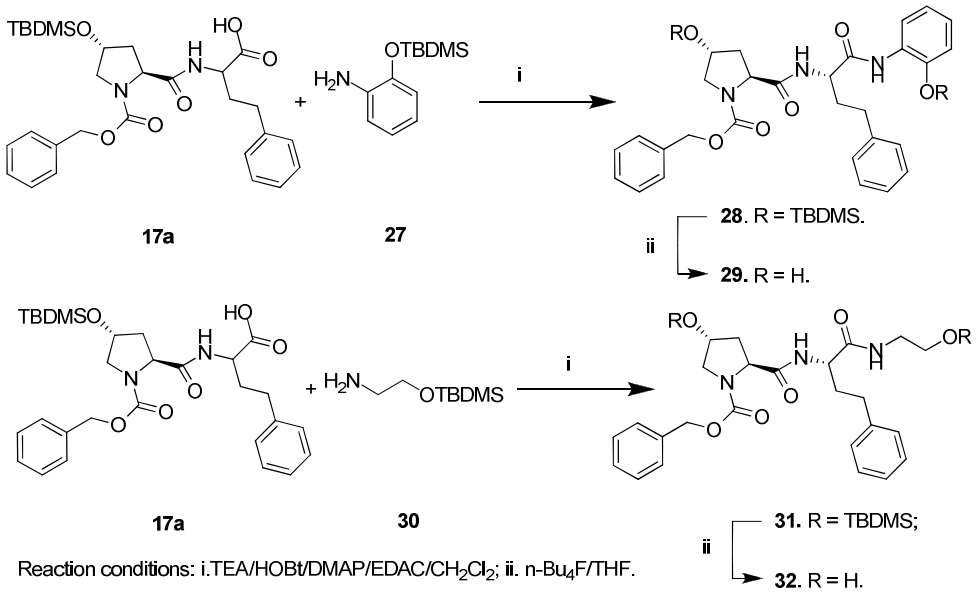
Peptidomimetic inhibitor **26** with cyano substituent directly attached to amide group was obtained from acid **14a** and 2-amino-4-phenylbutyronitrile hydrochloride **24** (Scheme 4).



Scheme 4. Synthesis of cyano-substituted peptidomimetic inhibitor **26**

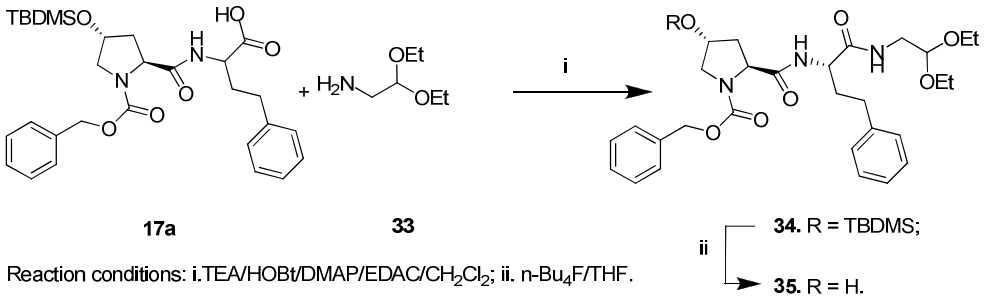
Requisite 2-amino-4-phenylbutyronitrile hydrochloride **24** was obtained from phenylpropionaldehyde by the known method [28].

The group of compounds bearing phenolic or aliphatic hydroxyl substituents and having ability for the formation of hydrogen bonds was designed and synthesized. TBDMS protected aminophenols **27** and 2-(tert-butyldimethylsilyloxy)ethylamine **30** were synthesized according to the standard procedure [27] from aminophenols or ethanolamine and TBDMS chloride in presence of imidazole or triethylamine in CH_2Cl_2. Reaction of **27**, **30** with acid **17a** gave derivatives **28**, **31** and the following removal of protected TBDMS groups led to the formation of desired peptidomimetics **29**, **32** (Scheme 5).



Scheme 5. Synthesis of phenolic and ethylenhydroxy substituted peptidomimetic inhibitors

For obtaining peptidomimetic bearing acetal function starting acid **17a** was reacted with commercially available aminoacetaldehyde diethylacetal **33** in standard conditions to give **34** and peptidomimetic derivative **35** was obtained after removing of the protected group (Scheme 6).



Scheme 6. Synthesis of aminoacetaldehyde diethylacetal peptidomimetic inhibitor **35**

Comparison of structure of lead compound **9** with known peptidomimetics and their mechanism of action brought to the idea that tri-peptides, bearing α-hydroxyketone residue with general structure **36** (Fig. 5) would have similar biological action. Due to potentialities inherent in variety of available α-aminoacid derivatives the great number of active peptidomimetics can be designed and synthesized.

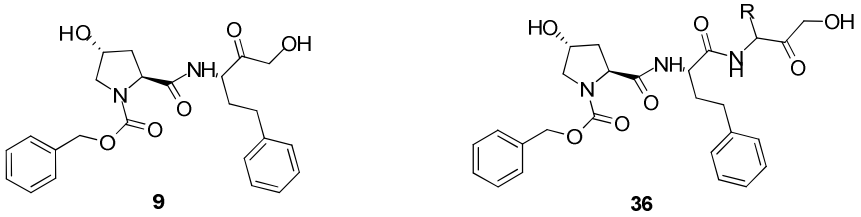
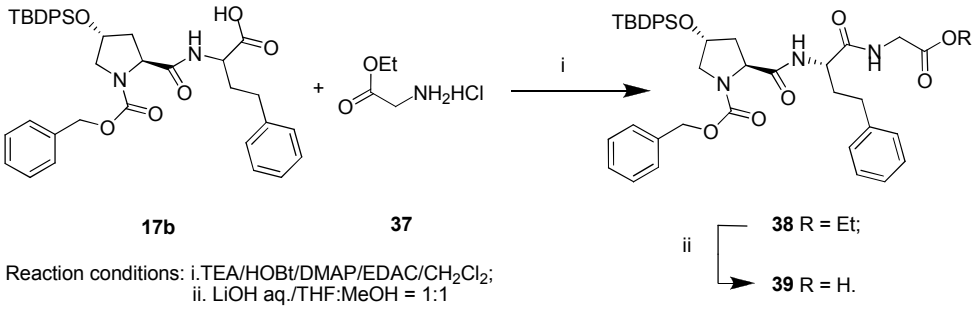


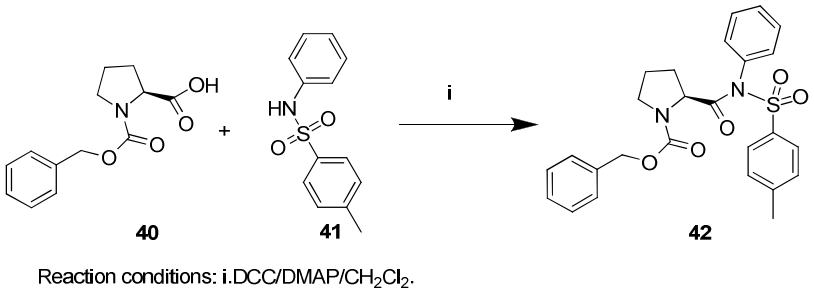
Figure 5. The lead peptidyl cysteine protease inhibitor and tri-peptides

For these purposes acid **17b** was reacted with glycine ethyl ester hydrochloride **37** to give desired tripeptide ester **38** which was saponified to acid **39** using LiOH (Scheme 7).



Scheme 7. Synthesis of peptidomimetic tripeptide ester **38**

We were planning to introduce (N-tosyl, N-aryl) fragments at P1 and P1'-sites of our prototypical molecule instead of the homophenylalanyl moiety, similar to the structures **3** and **4**. For this purpose, in the model reaction N-carbobenzyloxy-L-proline **40** was condensed with N-phenyl-4-toluensulfonamide **41** [29] to form the desired product **42** in good yield (Scheme 8).



Scheme 8. Model synthesis of peptidomimetic toluensulfonamide **42**

2.2 Biological results

There were about forty compounds bearing the various residues on the P'_1 site and P_2 site synthesized whose antimalaria activity was examined. Antimalarial activity was investigated as for the final peptide mimetics, and their immediate predecessors, carrying TBDMS or TBDPS protecting groups. The cytotoxicity of the peptidyl cysteine protease inhibitors against *P. falciparum* (D6 and W2 clones) is shown in Table 1. The *in vitro* growth inhibition of studied derivatives expressed as IC_{50} was within the range 1.1-7.4 µM/L (528-5280 ng/mL). About half of the synthesized compounds was not exhibited admissible antimalarial activity (IC_{50} over 5280 ng/mL) and was considered as inactive one.

Among active compounds, the derivatives, wherein the 4-hydroxyproline group was protected as the silyl ethers, namely substances **19**, **22**, **25**, **28**, **31**, **34**, **38** and **39**, were quantitatively dominated. Based on the obviously greater lipophilic the nature of silyl substituted molecules one can say that this clearly

observed fact can be attributed to an easier penetration of these derivatives into target cells [30] and probably their resistance to intracellular proteases. These considerations also suggest that as well as P_3 site, the P_2 site of inhibitor molecule must maintain a certain hydrophobicity to preserve the anti-plasmodium activity. Similar structure-activity relationships of potential choline or ethanolamine analogues aimed at increasing lipophilicity were essential for antimalarial activity and interfering with *P. falciparum* phospholipid metabolism [31].

Although the studied compounds were hydrophobic with a parameter LogP, calculated by various methods [32], in the range 1.8 – 9.3 (Table 1, only LogP values for Meylan/Kowwin model were shown), the acceptable correlation of these values with antiplasmodium activity was not found.

Among active substances silyl substituted derivatives were dominating, but the most active compound found was **23e** with the free hydroxyl group on proline residue and N-(cyanomethyl)-N-methylamino group at P'_1 site. Approximately 2.5 times less active agent in the studied row was derivative **31**, bearing TBDPS protecting groups on hydroxyl substituent group on the proline and the aminoethyl moieties. Another compound **32** with the free hydroxyl group on proline residue and 2-hydroxyphenyl substituent exhibited moderate activity. This finding is consistent with the hypothesis that the molecules bearing a hydroxyl group on the proline ring displayed an additional increase of activity [15]. On the other hand, the activity of the silyl derivatives can probably be attributed to their lipophilicity and easier penetration through cell membranes. Moreover, bulky substituent may facilitate proper orientation of the agent in the enzyme active site.

The data may indicate that for better bioavailability the inhibitor molecule must contain at P_3 site the low polar substituents. At the same time, the high activity undoubtedly suggests positive influence free hydroxyl group in the pyrrolidine ring. The data clearly revealed that the obtained results require a more thorough QSAR examination, which is currently ongoing. Results of obtained QSAR model will be published elsewhere.

Table 1. Inhibition of P. falciparum and LogP data of active compounds (**19-39**)

Compounds	P. falciparum (D6 clone) Inhibition			P. falciparum (W2 clone) Inhibition			LogP model
	IC_{50} (ng/mL)	IC_{50} (μM/L)	SI	IC_{50} (ng/mL)	IC_{50} (μM/L)	SI	Meylan/Kowwin [32]
19a	2700	4.1	>1.8	3200	4.9	>1.5	4.75
19b	2600	3.9	>1.8	2800	4.2	>1.7	3.60
19d	3200	4.8	>1.5	2800	4.2	>1.7	4.53
19e	4000	6.5	>1.2	4760	7.8	>1.0	5.64
19f	3000	4.0	>1.6	2000	2.7	>2.4	6.33
22b	2800	4.3	>1.7	1600	2.5	>3.0	7.50
22c	4200	6.6	>1.1	2800	4.4	>1.7	5.92

22d	4760	6.4	>1.0	4760	6.4	>1.0	2.76
22e	5280	7.4	>9.0	5280	7.4	>9.0	5.62
23e	528	1.1	>9.0	720	1.5	>6.6	7.51
24	3200	16.3	>1.5	2800	14.2	>1.7	9.09
25	2000	3.7	>2.4	1400	2.6	>3.4	3.01
28	3000	4.7	>1.6	3200	5.1	>1.5	2.32
31	1300	1.9	>3.7	1500	2.1	>3.2	3.67
32	2800	5.4	>1.7	3200	6.2	>1.5	2.41
34	3000	4.8	>1.6	3000	4.8	>1.6	3.00
38	2300	3.1	>2.1	2000	2.7	>2.4	1.84
39	3200	4.4	>1.5	4000	5.5	>1.2	1.84
Chloroquine		0.4			0.05		
artemisinin	8	0.017			0.0145		

Note 1. Compounds with IC_{50} over 5280 ng/mL were considered as inactive ones and were not included in Table 1.

Note 2. SI is a selectivity index: SI = CC_{50}/IC_{50}, where CC_{50} is a cytotoxic concentration. All these compounds were not cytotoxic to Vero cells.

2.3 Conclusions

In the summary, we designed and synthesized a new series of peptidomimetic N-substituted Cbz-4-Hyp-Hpa-amides as potential antimalarial agents. The study found that numerous peptidomimetics corresponding to the lead structure and the current model of the active antimalarial agents may be synthesized by condensation of protected Cbz-4-hydroxy-L-proline and L-homophenylalanine, followed by the addition of the variety of substituents to C-terminus L-homophenylalanine in order to create variations in P'_1 site.

The evaluation of antimalarial activity of the obtained compounds showed that the model generally corresponded to the structural requirements of the active compound. The analysis of the biological activity data showed that the substituent at the hydroxyl group of hydroxyproline could also affect the antimalarial activity of the peptidomimetic. In the study an acceptable correlation between calculated LogP values (Meylan/Kowwin model) with antiplasmodium activity was not found.

3. Experimental work

3.1. Chemistry

General Methods: All solvents and chemicals were purchased from Aldrich. Anhydrous solvents were stored with molecular sieves. THF, ether, and DME were distilled from sodium-benzophenone; acetonitrile and CH_2Cl_2 were distilled from P_2O_5 immediately prior to the use. Final inhibitors **19–39** were synthesized as detailed in the following procedures below. The reactions were conducted under argon atmosphere. Melting points were determined on a Fargo melting point apparatus and were not corrected. ^{1}H NMR and ^{13}C NMR spectra were determined on Bruker 400, 500 and 600 MHz spectrometers in $CDCl_3$ or DMSO-d_6 solution at 25 °C. Chemical shifts (δ) were expressed in ppm downfield from internal standard TMS. Infrared (IR) spectra were obtained on Bruker Vector33 FTIR spectrometer. Optical rotations were measured on Rudolph Autopol IV polarimeter. MS spectra were carried out on HP 5973 GC-MS instrument. High Resolution Mass spectra were recorded on Bruker BioApex LC-Mass system with a Lock Spray Source or with a FTMS system by direct injection using an electrospray interface (ESI). Flash column chromatography was carried out over silica gel G60 (70-230 mesh, ASTM; Merck). Thin layer chromatography (TLS) was performed on silica gel G60 F254 (Merck) plates with short wave length UV light for visualization. The purity of all final compounds was determined to be ≥95% by NMR and HPLC-MS analysis.

(2*S*,4*R*)-Benzyl 4-(tert-butyldimethylsilyloxy)-2-((*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-ylcarbamoyl)pyrrolidine-1-carboxylate (**16**). Triethylamine (TEA, 1.518 g, 2.08 mL, 15 mmol), hydroxybenzotriazole (HOBt, 0.811 g, 6 mmol), dimethylaminopyridine (DMAP, 6 mg, 0.05 mmol) in CH_2Cl_2 *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDAC, 1.15 g, 6 mmol) were added to the solution of (2*S*,4*R*)-1-(benzyloxycarbonyl)-4-(tert-butyl-dimethylsilyloxy)pyrrolidine-2-carboxylic acid (**14**, 1.897 g, 5 mmol) at -5 °C. A reaction mixture was stirred under Ar pad for 2 h at room temperature then ethyl (*S*)-2-amino-4-phenylbutyrate hydrochloride (*L*-homophenylalanine ethyl ester hydrochloride, **15**, 1.243 g, 5.1 mmol) was added and stirring was continued for 20 h. After this time the reaction mixture was mixed with saturated NH_4Cl solution (20 mL) at 0 °C, formed layers were separated, water solution was extracted with ethyl acetate (4×6 mL), organic phases were combined, washed with brine (10 mL), dried over Na_2SO_4, filtered and concentrated in vacuo to give a crude product (3.38 g), which was purified by column chromatography on SiO_2 (150 g, column 30×250 mm) using gradient ethyl acetate/hexanes = 10/90 to 30/70 as eluant affording 2.719 g (95.7%) of product **16** as syrup. $[\alpha]_D^{27.4}$ = -15.0 (c = 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.044, 0.060 (each 3H, s, 2×CH_3), 0.854 (9H, s, 3×CH_3), 1.15 (3H, br. t, CH_3), 1.52 (2H, m, CH_2), 1.80 (4H, m, 2×CH_2), 2.27 (1H, m, CH), 2.49 (2H, m, CH_2), 3.33 (2H, m, CH_2), 4.01 (2H, m, CH_2), 4.32 (3H, m, CH & CH_2), 5.01 (2H, m, CH_2), 6.30 (1H, br. s, NH), 7.1-7.4 (10H, m, PhH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.12, 171.37, 156.58, 155.49, 141.13, 140.82, 136.65, 128.70, 128.27, 128.04, 126.31, 77.68, 77.56, 77.36, 77.04, 70.75, 70.07, 67.58, 61.58, 60.56, 59.98, 59.55, 55.89, 54.95, 52.46, 52.20, 40.45, 37.55, 34.23, 31.74, 25.92, 21.22, 18.15, 14.39, -4.58, -4.69. IR (film) ν_{max} 418.2, 698.6, 748.1, 837.1, 1024.2, 1115.7, 1204.1, 1356.5, 1419.0, 1539.7, 1683.3, 2360.3, 2930.4, 3314.2 cm^{-1}. HRMS (TOF) (m/z): M+H$^+$ calcd for $C_{31}H_{45}N_2O_6Si$ 569.3059, found 569.3047.

2-((2*S*,4*R*)-1-(Benzyloxycarbonyl)-4-(tert-butyldimethylsilyloxy) pyrrolidine-2-carboxamido)-4-phenylbutanoic acid (**17a**). Aqueous solution of LiOH (1 M) was added dropwise to the solution of **16** (2.273 g, 4 mmol) in THF/MeOH (1/1, 20 mL) with stirring at -5 °C. After 3.5 h turbid reaction mixture became transparent and after 4 h TLC analysis was shown consuming of starting ester. The reaction mixture was cooled in ice-water bath and acidified with 1 N HCl to *pH* ~5.5, then extracted with EtOAc (5×5 mL). Organic phases were combined, washed with brine (5 mL), water (5 mL), dried over Na_2SO_4, filtered and

concentrated in vacuo to give a product (**17a**), 1.793 g (82.9%) which was pure for using in the next step. Mp 119 °C. $[\alpha]_D^{22.1}$ = -18.4 (c = 1, MeOH); $[\alpha]_D^{27.4}$ = -15.4(c = 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.040, 0.055 (each 3H, s, 2×CH_3), 0.85 (9H, s, 3×CH_3), 1.98 (2H, m, CH_2), 2.18 (1H, m, CH), 2.33 (1H, m, CH), 2.62 (2H, m, CH_2), 3.43 (1H, m, CH), 3.56 (1H, m, CH), 4.50 (3H, m, CH & CH_2), 5.13 (2H, m, CH_2), 6.55 (1H, br. s, NH), 7.1-7.4 (10H, m, PhH). IR (film) ν_{max} 426.0, 441.1, 698.2, 836.8, 1022.4, 1116.3, 1172.9, 1254.1, 1357.0, 1420.4, 1537.1, 1684.8, 2929.4 cm^{-1}. HRMS (TOF) (m/z): M+H$^+$ calcd for $C_{29}H_{40}N_2O_6Si$ 541.2716, found 541.2734.

(2*S*,4*R*)-Benzyl 4-(tert-butyldimethylsilyloxy)-2-(1-oxo-4-phenyl-1-(2-(phenylamino)ethyl-amino)butan-2-ylcarbamoyl)pyrrolidine-1-carboxylate (**19a**) and **(2*S*,4*R*)-benzyl 2-(1-((2-aminoethyl)(phenyl)amino)-1-oxo-4-phenylbutan-2-ylcarbamoyl)-4-(tert-butyldimethylsilyloxy)-pyrrolidine-1-carboxylate** (**19b**). *General procedure.* TEA (152 mg, 0.208 mL, 1.5 mmol), HOBt (81 mg, 0.6 mmol), DMAP (0.6 mg, 0.05 mmol) in CH_2Cl_2 (5 mL) EDAC (115 mg, 0.6 mmol) was added to the solution of 2-((2*S*,4*R*)-1-(benzyloxycarbonyl)-4-(tert-butyldimethylsilyloxy)pyrrolidine-2-carboxamido)-4-phenylbutanoic acid (**17a**, 270 mg, 0.5 mmol)at -5 °C. A reaction mixture was stirred under Ar pad for 0.5 h at room temperature then N-phenylethylenediamine (**18a**, 82 mg, 0.079 mL, 0.6 mmol) was added and stirring was continued for 22 h. After this time the reaction mixture was mixed with saturated NH_4Cl solution (2 mL) at 0 °C, formed layers were separated, water solution was extracted with ethyl acetate (4×2 mL), organic phases were combined, washed with brine (2 mL), dried over Na_2SO_4, filtered and concentrated in vacuo to give a crude product which was purified by column chromatography on SiO_2 (18 g, column 15×220 mm) using gradient ethyl acetate/hexanes = 10/90 to 30/70 as eluant yielding two products, **19a** 67 mg (20.4%) and **19b** 49 mg (14.9%) as syrups. **19a** $[\alpha]_D^{26.4}$=-30.4 (c=1, $CHCl_3$) and **19b** $[\alpha]_D^{26.3}$=-27.2 (c=1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$); **19a**: δ 0.060, 0.081 (each 3H, s, 2×CH_3), 0.880 (9H, s, 3×CH_3), 2.00 (2H, m, CH_2), 2.16 (1H, m, CH), 2.35 (1H, m, CH), 2.68 (2H, m, CH_2), 3.26 (3H, m, CH & CH_2), 3.44 (2H, m, CH_2), 3.60 (2H, m, CH_2), 4.09 (1H, t, J = 8.4 Hz, CH), 4.44 (2H, m, CH_2), 4.96 (1H, d, J = 12.8 Hz, CH), 5.05 (1H, d, J = 12.8 Hz, CH), 6.30 (1H, br. s, NH), 6.69 (3H, m, 3×CH, PhH), 7.12-7.32 (12H, m, PhH), 7.51 (2H, s, NH). HRMS (TOF) (m/z): M+H$^+$ calcd for $C_{37}H_{51}N_4O_5Si$ 659.3629, found 659.3656. **19b**: δ 0.06, 0.08 (each 3H, s, 2×CH_3), 0.88 (9H, s, 3×CH_3), 2.00 (2H, m, CH_2), 2.16 (1H, m, CH), 2.35 (1H, m, CH), 2.68 (2H, m, CH_2), 3.26 (3H, m, CH & CH_2), 3.44 (2H, m, CH_2), 3.60 (2H, m, CH_2), 4.09 (1H, t, J = 8.4 Hz, CH), 4.44 (2H, m, CH_2), 4.96 (1H, d, J = 12.8 Hz, CH), 5.05 (1H, d, J = 12.8 Hz, CH), 6.30 (1H, br. s, NH), 6.69 (3H, m, 3×CH, PhH), 7.12-7.32 (12H, m, PhH), 7.51 (2H, s, NH). IR (film) **19a** ν_{max} 428.7, 467.1, 693.4, 746.8, 780.1, 842.5, 901.5, 1020.1, 1115.6, 1171.4, 1205.2, 1254.3, 1358.9, 1417.7, 1513.4, 1604.8, 1653.5, 2360.9, 2857.5, 2927.3, 3021.6, 3302.4 cm^{-1}; **19b** ν_{max} 426.6, 457.0, 695.7, 748.6, 782.3, 836.9, 900.7, 1022.2, 1115.8, 1171.3, 1206.0, 1256.8, 1356.1, 1418.0, 1512.6, 1603.6, 1650.4, 2360.5, 2856.2, 2928.6, 3028.7, 3300.6 cm^{-1}. HRMS (TOF) (m/z) **19a**: M+H$^+$ calcd for $C_{37}H_{51}N_4O_5Si$ 659.3629, found 659.3648; **19b**: M+H$^+$ calcd for $C_{37}H_{51}N_4O_5Si$ 659.3629, found 659.3611.

(2*S*,4*R*)-Benzyl 4-(tert-butyldimethylsilyloxy)-2-(1-oxo-4-phenyl-1-(2-(p-tolylamino)ethyl-amino)butan-2-ylcarbamoyl)pyrrolidine-1-carboxylate (**19c**) and **(2*S*,4*R*)-benzyl 2-(1-((2-aminoethyl)(p-tolyl)amino)-1-oxo-4-phenylbutan-2-ylcarbamoyl)-4-(tert-butyldimethylsilyloxy)pyrrolidine-1-carboxylate** (**19d**) were synthesized following general procedure from **17a** (108 mg, 0.2 mmol) and appropriate **18b** [33] (31 mg, 0.205 mmol). It was isolated 37 mg (27.5%) **19c** and 24 mg (17.8%) of **19d** as syrups. **19c** $[\alpha]_D^{26.2}$ = 37.4 (c=1, $CHCl_3$), **19d** $[\alpha]_D^{25.9}$ = 58.7 (c=0.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$); **19c**: δ 0.031, 0.046 (each 3H, s, 2×CH_3), 0.845 (9H, s, 3×CH_3), 2.02 (4H, m, 2×CH_2), 2.26 (3H, s, CH_3), 2.36 (1H, m, CH), 2.64 (2H, m, CH_2), 3.20 (1H, m, CH), 3.37 (2H, m, CH_2), 3.48 (3H, m, CH & CH_2), 3.62 (2H, m, CH_2), 4.41 (3H, m, CH & CH_2), 4.96 (1H, d, J = 12.8 Hz, CH), 5.06 (1H, d, J = 12.8 Hz, CH), 5.16 (1H, d, J = 12.8 Hz, CH), 6.43 (1H, br. s, NH), 6.73 (1H, br. s, NH), 7.03-7.47 (15H, m, PhH), 7.47 (1H, s, NH). HRMS (TOF) (m/z): M+H$^+$ calcd for $C_{38}H_{53}N_4O_5Si$ 673.3791, found 673.3824. **19d**: δ 0.06, 0.08 (each 3H, s, 2×CH_3), 0.88 (9H, s, 3×CH_3), 1.26 (3H, s, CH_3), 2.01 (2H, m, CH_2), 2.22 (2H, m, CH), 2.36 (2H, m, CH_2), 2.69 (1H, m, CH_2), 3.34 (4H, m, 2×CH & CH_2), 3.62 (1H, m,

CH_2), 4.16 (1H, t, J = 8.4 Hz, CH), 4.49 (2H, m, CH_2), 4.92 (1H, d, J = 12.4 Hz, CH), 5.06 (1H, d, J = 12.4 Hz, CH), 6.77 (2H, br. s, NH_2), 6.98-7.28 (15H, m, PhH), 7.63 (1H, s, NH). IR (film) **19c** ν_{max} 412.8, 698.2, 777.5, 807.9, 836.5, 1022.3, 1116.0, 1169.7, 1257.0, 1355.9, 1424.0, 1522.4, 1680.4, 2856.4, 2927.9, 3340.4cm^{-1}; **19d** ν_{max} 413.1, 418.7, 425.1, 430.2, 434.2, 441.9, 447.1, 458.1, 477.1, 699.3, 1126.2, 1357.3, 1429.8, 1513.5, 1669.4, 2924.5, 3316.4 cm^{-1}. HRMS (TOF) (m/z) **19c**: $M+H^+$ calcd for $C_{38}H_{53}N_4O_5Si$ 673.3824, found 673.3801; **19d**: $M+H^+$ calcd for $C_{38}H_{53}N_4O_5Si$ 673.3785, found 673.3801.

(2*S*,4*R*)-Benzyl 4-(tert-butyldimethylsilyloxy)-2-(1-(2-(dimethylamino)ethylamino)-1-oxo-4-phenylbutan-2-ylcarbamoyl)pyrrolidine-1-carboxylate (**19e**) was synthesized following general procedure from **17a** (270 mg, 0.5 mmol) and **18e** (53 mg, 0.6 mmol) affording 230 mg (75.3%) **19e** as syrup. $[\alpha]_D^{27.4}$ = -10.8 (c = 0.5, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 0.034, 0.054 (each 3H, s, 2×CH_3), 0.85 (9H, s, 3×CH_3), 2.13 (2H, m, CH_2), 2.29 (1H, m, CH), 2.47 (1H, m, CH_2), 2.67 (2H, m, CH_2), 3.18 (3H, m, CH & CH_2), 3.32 (1H, m, CH_2), 3.46 (2H, m, CH_2), 3.61 (2H, m, CH_2), 4.41 (2H, m, CH_2), 5.15 (2H, dd, J = 11.6 Hz, CH_2), 6.22 (1H, s, NH), 6.9-7.5 (12H, m, PhH & 2×NH). IR (film) ν_{max} 410.0, 418.4, 428.9, 641.0, 698.9, 749.1, 891.5, 1087.6, 1244.1, 1312.1, 1356.5, 1448.7, 1570.2, 1629.2, 2851.4, 2927.7, 3329.3 cm^{-1}. HRMS (TOF) (m/z): $M+H^+$ calcd for $C_{33}H_{51}N_4O_5Si$ 611.3629, found 611.3617.

(*2S,4R*)-Benzyl 4-(tert-butyldiphenylsilyloxy)-2-((*S*)-1-((2-(dimethylamino)ethyl)-(methyl)amino)-1-oxo-4-phenylbutan-2-ylcarbamoyl)pyrrolidine-1-carboxylate (**19f**) was synthesized following general procedure from **17b** (200 mg, 0.3 mmol, $[\alpha]_D^{22.3}$ = -26.4 (c = 1, $CHCl_3$)) and **18f** (37 mg, 0.36 mmol) affording 166 mg (73.9%) **19f** as syrup. $[\alpha]_D^{27.4}$ = -8.4 (c = 0.5, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 7.65 – 6.95 (m, 21H), 5.13 (m, 2H), 4.75 (m, 1H), 4.56 (m, 1H), 4.39 (m, 1H), 3.43 (s, 5H), 2.87 (m, 4H), 2.72 – 2.32 (m, 8H), 2.24 (m, 3H), 1.98 (m, 4H), 1.00 (s, 9H). IR (film) ν_{max} 3293.4, 418.4, 430.3, 447.6, 464.5, 508.1, 611.7, 701.5, 743.1, 822.2, 1022.4, 1112.1, 1169.3, 1356.1, 1416.8, 1455.1, 1496.4, 1640.2, 1706.4, 2360.5, 2857.5, 2931.1 cm^{-1}. HRMS (TOF) (m/z): $M+H^+$ calcd for $C_{44}H_{57}N_4O_5Si$ 749.4098, found 749.4115.

(2*S*,4*R*)-Benzyl 4-hydroxy-2-((*S*)-1-oxo-4-phenyl-1-(2-(phenylamino)ethylamino)butan-2-ylcarbamoyl)pyrrolidine-1-carboxylate (20a). *General procedure.* n-Bu_4NF (1M solution in THF, 0.2 mL) was added to stirred solution of **19a** (66 mg, 0.1 mmol) in THF (1 mL) and the reaction mixture was stirred at room temperature. After 1 h reaction was finished, brine (2 mL) was added, formed layers were separated, water solution was extracted with EtOAc (3×1 mL), and organic phases were combined, dried over Na_2SO_4, filtered and concentrated in vacuo. A residue was purified with column chromatography on SiO_2 (10 g, column 15×210 mm), using $CH_3OH/CHCl_3$ = 5/95, 10/90 (v/v) as eluant yielding 43.5 mg of **20a** (79.8%). Mp 115 °C. $[\alpha]_D^{26.2}$ = -32.4 (c = 0.5, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 7.78 – 6.46 (m, 17H), 5.08 (s, 2H), 4.69 (m, 1H), 4.40 (m, 3H), 3.44 (m, 7H), 2.97 (m, 1H), 2.76 – 2.39 (m, 2H), 2.19 (m, 2H), 2.00 (m, 2H), 1.75 (m, 1H), 1.46 – 1.13 (m, 2H). IR (film) ν_{max} 425.4, 696.1, 750.0, 1126.3, 1356.9, 1422.2, 1511.7, 1603.2, 1651.2, 2927.3, 3306.2 cm^{-1}. HRMS (TOF, m/z): $M+H^+$ calcd for $C_{31}H_{37}N_4O_5$ 545.2764, found 545.2777.

(2*S*,4*R*)-Benzyl 2-(1-((2-aminoethyl)(phenyl)amino)-1-oxo-4-phenylbutan-2-ylcarbamoyl)-4-hydroxypyrrolidine-1-carboxylate (**20b**) was synthesized according to general procedure from **19b** (44 mg) and n-Bu_4NF (1M solution in THF, 0.14 mL) in anh. THF (1 mL), yield was 28 mg (60.3%). Mp 114 °C (dec.). $[\alpha]_D^{26.8}$ = -32.4 (c = 1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 1.92 (2H, m, CH_2), 2.08-2.30 (3H, m, 3×CH), 2.66 (2H, m, CH_2), 2.99 (1H, m, CH), 3.09 – 3.46 (5H, m, 5×CH), 3.52 (1H, d, J = 11.6 Hz, CH), 3.61 (1H, dd, J = 3.6, 11.6 Hz, CH), 4.20 (1H, m, CH), 4.33 (1H, t, J = 8.0 Hz, CH), 4.46 (1H, br.s, CH), 5.05 (2H, dd, J = 12.4 & 13.3 Hz, CH_2), 6.60 (3H, m, PhH & OH), 7.05-7.40 (13H, m, PhH & 2×NH), 7.05-7.53 (1H, m, NH). IR (film) ν_{max} 418.4, 434.2, 696.4, 749.8, 1129.3, 1173.0, 1357.6, 1430.0, 1539.1, 1603.3, 1673.2, 2925.4, 3352.2 cm^{-1}. HRMS (TOF) (m/z): $M+H^+$ calcd for $C_{31}H_{37}N_4O_5$ 545.2764, found 545.2770.

(*2S,4R*)-Benzyl 4-hydroxy-2-((*S*)-1-oxo-4-phenyl-1-(2-(*p*-tolylamino)ethylamino)butan-2-ylcarbamoyl)pyrrolidine-1-carboxylate (**20c**) was synthesized according to general procedure from **19c** (22 mg) and n-Bu_4NF (1M solution in THF, 0.065 mL) in anh. THF (0.5 mL), yield was 17.2 mg (96.1%). Mp 134 °C. $[\alpha]_D^{26.7}$ = -19.4 (c = 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (s, 1H), 7.23 – 6.91 (m, 14H), 6.85 (s, 1H), 6.75 (d, J = 8.1, 2H), 6.40 (d, J = 7.5, 2H), 4.88 (s, 3H), 4.30 (s, 2H), 4.25 – 4.06 (m, 3H), 3.54 – 3.15 (m, 6H), 2.76 (s, 1H), 2.47 (s, 2H), 2.31 (s, 1H), 2.15 (s, 2H), 2.02 (s, 4H), 1.87 (s, 1H), 1.80 (s, 2H), 1.59 (s, 1H). IR (film) ν_{max} 418.1, 699.5, 741.4, 1124.8, 1172.3, 1356.6, 1419.5, 1521.7, 1681.0, 2961.0, 3306.6 cm^{-1}. HRMS (TOF) (m/z): $M+H^+$ calcd for $C_{31}H_{37}N_4O_5$ 545.2764, found 545.2770.

(*2S,4R*)-Benzyl 2-((*S*)-1-(2-(dimethylamino)ethylamino)-1-oxo-4-phenylbutan-2-ylcarbamoyl)-4-hydroxypyrrolidine-1-carboxylate (**20e**) was synthesized according to general procedure from **19e** (158 mg) and n-Bu_4NF (1M solution in THF, 0.52 mL) in anh. THF (0.5 mL), yield was 111 mg (90.4%). Mp 136 °C. $[\alpha]_D^{26.0}$ = 9.2 (c = 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (d, J = 6.0, 1H), 8.03 (s, 1H), 7.41 – 7.05 (m, 10H), 5.30 (s, 1H), 5.06 (s, 2H), 4.59 (m, 3H), 3.83 (m, 1H), 3.67 (m, 2H), 3.01 (m, 2H), 2.67 (m, 2H), 2.62 – 2.51 (m, 5H), 2.41 (d, J = 6.6, 2H), 2.27 (m, 3H), 2.16 – 1.96 (m, 1H). IR (film) ν_{max} 415.9, 432.7, 446.5, 700.2, 746.1, 1124.0, 1170.2, 1357.4, 1423.5, 1537.6, 1678.0, 2960.0, 3333.4 cm^{-1}. HRMS (TOF) (m/z): $M+H^+$ calcd for $C_{27}H_{37}N_4O_5$497.2764, found 497.2759.

(*2S,4R*)-Benzyl 2-((*S*)-1-((2-(dimethylamino)ethyl)(methyl)amino)-1-oxo-4-phenylbutan-2-ylcarbamoyl)-4-hydroxypyrrolidine-1-carboxylate (**20f**) was synthesized according to general procedure from **19f** (28 mg) and n-Bu_4NF (1M solution in THF, 0.075 mL) in anh. THF (0.5 mL), yield was 17.6 mg (92.7%) as a syrup. $[\alpha]_D^{26.0}$ = -20.1 (c = 2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 6.93 (m, 11H), 5.10 (m, 2H), 4.86 (m, 1H), 4.74 – 4.33 (m, 3H), 3.65 (m, 3H), 3.04 – 2.72 (m, 3H), 2.69 – 2.22 (m, 8H), 2.22 – 2.05 (m, 3H), 1.95 (m, 2H), 1.23 (m, 3H). IR (film) ν_{max} 413.0, 431.5, 444.3, 455.2, 700.0, 740.2, 1120.4, 1170.1, 1231.6, 1355.9, 1417.0, 1456.3, 1490.0, 1541.4, 1638.3, 1702.3, 2874.6, 2960.5, 3286.3 cm^{-1}. HRMS (TOF) (m/z): $M+H^+$ calcd for $C_{28}H_{39}N_4O_5$511.2920, found 511.2930.

(*2S,4R*)-Benzyl 4-(tert-butyldimethylsilyloxy)-2-((*S*)-1-(2-cyanoethylamino)-1-oxo-4-phenylbutan-2-ylcarbamoyl)pyrrolidine-1-carboxylate (**22a**) was synthesized following general procedure from **17a** (162 mg, 0.3 mmol) and **21a** (25 mg, 0.36 mmol) yielding 117 mg (65.8%) **22a**. Mp 167 °C.$[\alpha]_D^{25.9}$ = -24.0 (c = 0.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (s, 1H), 7.49 – 7.07 (m, 13H), 6.41 (d, *J* = 7.7, 1H), 6.22 (d, *J* = 9.4, 1H), 5.42 – 4.93 (m, 2H), 4.63 – 4.19 (m, 3H), 4.08 (t, *J* = 7.5, 1H), 3.87 – 3.29 (m, 4H), 3.27 – 3.02 (m, 1H), 2.82 – 2.49 (m, 3H), 2.46 (m, 1H), 2.16 (m, 2H), 2.06 – 1.91 (m, 2H), 1.83 (m, 3H), 0.85 (s, 9H), 0.06 (s, 6H). IR (film) ν_{max} 412.4, 418.8, 424.5, 430.0, 433.2, 437.6, 442.9, 451.7, 466.6, 474.3, 699.0, 1124.7, 1172.6, 1357.4, 1421.7, 1546.2, 1666.7, 2926.0, 3301.2 cm^{-1}. HRMS (TOF) (m/z): $M+H^+$ calcd for $C_{32}H_{45}N_4O_5Si$ 593.3159, found 593.3154.

(*2S,4R*)-Benzyl 2-((*S*)-1-(bis(2-cyanoethyl)amino)-1-oxo-4-phenylbutan-2-ylcarbamoyl)-4-(tert-butyldimethylsilyloxy)pyrrolidine-1-carboxylate (**22b**) was synthesized by following general procedure from **17a** (162 mg, 0.3 mmol) and **21b** (49 mg, 0.4 mmol) affording 101 mg (52.3%) **22b** as syrup. $[\alpha]_D^{27.4}$ = -20.0 (c = 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.042, 0.058 (each 3H, s, 2×CH_3), 0.85 (9H, s, 3×CH_3), 1.83 (2H, m, CH_2), 2.00 (2H, m, CH_2), 2.20 (1H, m, CH), 2.41 (1H, m, CH), 2.56 (1H, m, CH), 2.70 (2H, m, CH_2), 3.19 (1H, m, CH), 3.36 (1H, m, CH), 3.57 (3H, m, CH & CH_2), 4.41 (3H, m, CH & CH_2), 5.19 (2H, m, CH_2), 6.41 (1H, d, J = 9.6 Hz, NH), 7.19-7.5 (10H, m, PhH), 7.78 (1H, s, NH). IR (film) ν_{max} 410.7, 414.6, 572.4, 700.9, 751.8, 837.3, 1126.1, 1171.3, 1203.0, 1357.7, 1421.8, 1497.7, 1680.0, 2249.2, 2348.8, 2929.8, 3338.7 cm^{-1}. HRMS (TOF) (m/z): M-($C_3H_3N)_2$+H^+ calcd for $C_{29}H_{43}N_3O_5Si$ 541.2972, found 541.3139.

(2*S*,4*R*)-Benzyl 2-(1-(allyl(2-cyanoethyl)amino)-1-oxo-4-phenylbutan-2-ylcarbamoyl)-4-(tert-butyldimethylsilyloxy)pyrrolidine-1-carboxylate (**22c**) was synthesized following general procedure from **17a** (162 mg, 0.3 mmol) and **21c**, obtained according to [34] (40 mg, 0.36 mmol) affording 157 mg (82.7%) **22c** as syrup. $[\alpha]_D^{27.4}$ = -36.8 (c = 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm) 0.044, 0.06 (each 3H, s,

2×CH_3), 0.85 (9H, s, 3×CH_3), 1.91 (3H, m, CH & CH_2), 2.25 (3H, m, CH& CH_2), 2.59 (4H, m, 4×CH), 3.16 (1H, m, CH), 3.53 (4H, m, 4×CH), 3.98 (2H, m, 2×CH), 4.44 (2H, m, CH_2), 4.83 (1H, s, CH), 5.19 (4H, m, 4×CH), 5.66 (1H, br. s, NH), 7.2-7.5 (10H, m, PhH). IR (film) ν_{max} 416.9, 427.7, 436.9, 668.8, 699.3, 741.8, 995.7, 1121.9, 1172.4, 1233.1, 1357.3, 1419.3, 1497.5, 1540.4, 1647.8, 1698.8, 2341.5, 2360.4, 2927.8, 3309.8 cm^{-1}. HRMS (TOF) (m/z): $M+H^+$ calcd for $C_{35}H_{49}N_4O_5Si$ 633.3472, found 633.3501.

(***2S,4R***)**-Benzyl 4-(tert-butyldimethylsilyloxy)-2-((*S*)-1-(N-(2-cyanoethyl)-4-methylphenylsulfonamido)-1-oxo-4-phenylbutan-2-ylcarbamoyl)pyrrolidine-1-carboxylate** (**22d**) was prepared according to general procedure from **17a** (162 mg, 0.3 mmol) and **21d** (65 mg, 0.31 mmol). It yielded 103 mg (46.0%) **22d** as syrup. $[\alpha]_D^{25.9}$ = -28.7 (c = 0.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, *J* = 8.2, 2H), 7.76 (d, *J* = 8.3, 1H), 7.50 – 6.92 (m, 14H), 6.50 – 6.22 (m, 1H), 5.45 (m, 1H), 5.19 (m, 2H), 4.41 (m, 2H), 3.96 (m, 1H), 3.65 (m, 2H), 3.49 (m, 2H), 3.25 (m, 1H), 2.80 – 2.50 (m, 5H), 2.44 (m, 4H), 2.19 (m, 3H), 2.10 – 1.77 (m, 4H), 1.73 (m, 1H), 1.60 (m, 1H), 1.44 – 1.05 (m, 4H), 0.85 (s, 9H), 0.06 (2×s, 6H). IR (film) ν_{max} 412.9, 419.7, 424.0, 428.0, 433.3, 436.9, 441.1, 446.2, 453.0, 459.4, 548.5, 666.0, 699.8, 750.0, 814.7, 1091.7, 1163.3, 1358.7, 1419.9, 1540.2, 1684.8, 2927.8, 3337.9 cm^{-1}. HRMS (TOF) (m/z): $M+Na^+$ calcd for $C_{26}H_{27}N_2O_5SNa$769.3067, found 769.3033.

(***2S,4R***)**-Benzyl 4-(tert-butyldiphenylsilyloxy)-2-((*S*)-1-((cyanomethyl)(methyl)amino)-1-oxo-4-phenylbutan-2-ylcarbamoyl)pyrrolidine-1-carboxylate** (**22e**) was synthesized following general procedure from **17b** (200 mg, 0.3 mmol) and **21e** (39 mg, 0.36 mmol) affording 183 mg (84.9%) **22e** as syrup. $[\alpha]_D^{26.0}$ = -13.2 (c = 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, DMSO) δ 7.81 – 6.87 (m, 21H), 5.31 – 4.99 (m, 2H), 4.93 – 4.66 (m, 1H), 4.62 – 4.34 (m, 3H), 4.28 – 4.16 (m, 1H), 4.14 – 4.02 (m, 1H), 3.78 – 3.65 (m, 2H), 3.66 – 3.51 (m, 1H), 3.49 – 3.24 (m, 1H), 3.06 – 2.77 (m, 3H), 2.71 – 2.55 (m, 1H), 2.55 – 2.36 (m, 1H), 2.35 – 2.09 (m, 2H), 2.05 (m, 1H), 2.01 – 1.79 (m, 2H), 1.78 – 1.59 (m, 1H), 1.26 (m, 3H), 1.02 (s, 9H). IR (film) ν_{max} 419.8, 431.0, 439.5, 452.8, 458.5, 482.7, 612.8, 668.4, 701.2, 741.6, 822.2, 1022.1, 1112.6, 1169.5, 1265.5, 1355.0, 1416.2, 1653.5, 1701.1, 2360.0, 2857.4, 2930.4, 3313.9 cm^{-1}. HRMS (TOF) (m/z): $M+H^+$ calcd for $C_{42}H_{49}N_4O_5Si$ 717.3472, found 717.3456.

(***2S,4R***)**-Benzyl 2-((*S*)-1-((cyanomethyl)(methyl)amino)-1-oxo-4-phenylbutan-2-ylcarbamoyl)-4-hydroxypyrrolidine-1-carboxylate** (**23e**) was obtained following general procedure from **22e** (143 mg, 0.2 mmol) and n-Bu_4NF (1M solution in THF, 0.28 mL) in anh. THF (2 mL) to get after column chromatography 91 mg (95.1%) **23e**. Mp 169 °C. $[\alpha]_D^{26.0}$ = -18.8 (c = 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, DMSO) δ 7.82 – 6.88 (m, 11H), 5.31 – 4.98 (m, 2H), 4.94 – 4.64 (m, 1H), 4.60 – 4.33 (m, 3H), 4.28 – 4.16 (m, 1H), 4.13 – 4.02 (m, 1H), 3.75 – 3.64 (m, 2H), 3.66 – 3.51 (m, 1H), 3.49 – 3.24 (m, 1H), 3.06 – 2.77 (m, 3H), 2.71 – 2.55 (m, 1H), 2.55 – 2.36 (m, 1H), 2.35 – 2.09 (m, 2H), 2.05 (m, 1H), 2.01 – 1.79 (m, 2H), 1.78 – 1.59 (m, 1H), 1.26 (m, 3H). IR (film) ν_{max} 419.8, 431.8, 439.6, 452.4, 458.4, 482.9, 612.1, 668.2, 701.7, 741.3, 822.5, 1021.1, 1113.6, 1168.5, 1265.5, 1355.0, 1414.2, 1652.4, 1703.0, 2361.4, 2856.8, 2930.3, 3318.1 cm^{-1}. HRMS (TOF) (m/z): $M+H^+$ calcd for $C_{26}H_{30}N_4O_5$478.5407, found 478.5402.

(***2S,4R***)**-Benzyl 2-((*S*)-1-(2-cyanoethylamino)-1-oxo-4-phenylbutan-2-ylcarbamoyl)-4-hydroxypyrrolidine-1-carboxylate** (**23a**) was obtained following general procedure from **22a** (117 mg, 0.2 mmol) and n-Bu_4NF (1M solution in THF, 0.28 mL) in anh. THF (2 mL). Column chromatography purification yielded 20 mg (21.8%) **23a**. Mp 179 °C. $[\alpha]_D^{26.3}$ = -12.8 (c = 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (m, 1H), 7.27 (m, 10H), 5.13 (s, 2H), 4.39 (m, 3H), 3.97 – 3.41 (m, 19H), 3.36 (s, 1H), 3.24 (m, 1H), 2.81 – 2.42 (m, 4H), 2.30 (m, 1H), 2.18 (m, 2H), 1.95 (m, 1H), 1.69 (m, 1H), 1.26 (m, 1H). IR (film) ν_{max} 431.2, 445.6, 474.2, 700.4, 999.0, 1087.5, 1132.6, 1202.3, 1356.0, 1436.3, 1542.6, 1638.2, 1660.7, 1680.8, 2360.8, 2938.5, 3394.8 cm^{-1}. HRMS (TOF) (m/z): $M+H^+$ calcd for $C_{26}H_{31}N_4O_5$479.2294, found 479.2281.

(***2S,4R***)**-Benzyl 2-((*S*)-1-(allyl(2-cyanoethyl)amino)-1-oxo-4-phenylbutan-2-ylcarbamoyl)-4-hydroxypyrrolidine-1-carboxylate** (**23c**) was synthesized following general procedure from **22c** (143 mg, 0.3 mmol) and n-Bu_4NF (1M solution in THF, 0.34 mL) in anh. THF (2 mL), yield was 70 mg (59.7%). Mp

163 °C. $[\alpha]_D^{26.3}$ = -41.2 (c = 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 – 6.73 (m, 11H), 5.64 (m, 1H), 5.15 (m, 4H), 4.79 (m, 1H), 4.48 (m, 2H), 3.98 (m, 1H), 3.67 (m, 5H), 3.11 (m, 1H), 3.02 – 2.37 (m, 6H), 2.19 (m, 2H), 1.95 (m, 2H), 1.32 (m, 1H), 1.00 (m, 1H). IR (film) ν_{max} 417.3, 447.6, 699.8, 1122.4, 1357.2, 1419.1, 1634.1, 2926.6, 3306.7 cm^{-1}. HRMS (TOF) (m/z): $M+H^+$ calcd for $C_{29}H_{35}N_4O_5$519.2607, found 519.2599.

(*2S,4R*)-Benzyl 4-(tert-butyldimethylsilyloxy)-2-(1-cyano-3-phenylpropylcarbamoyl)pyrrolidine-1-carboxylate (**25**) was synthesized following general procedure from **14** (190 mg, 0.5 mmol) and **24** [28] (108 mg, 0.55 mmol). It isolated 102 mg (39.2%) **25** as syrup. $[\alpha]_D^{26.0}$ = -40.8 (c = 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, *J* = 25.2, 1H, NH), 7.46 – 6.97 (m, 10H, PhH), 5.29 – 5.04 (m, 2H, CH_2), 4.84 – 4.65 (m, 1H), 4.55 – 4.27 (m, 2H), 3.58 – 3.34 (m, 2H), 2.89 – 2.60 (m, 2H), 2.59 – 2.33 (m, 1H), 2.20 – 1.88 (m, *J* = 30.4, 16.5, 3H), 0.85 (s, 9H, t-Bu), 0.06 (2×s, 6H). IR (film) ν_{max} 410.8, 415.2, 426.6, 433.9, 438.4, 698.6, 750.3, 1120.3, 1172.4, 1356.6, 1422.4, 1531.9, 1682.8, 2928.6, 3306.8 cm^{-1}. HRMS (TOF) (m/z): $M+H^+$ calcd for $C_{29}H_{39}N_3NaO_4Si$ 544.2608, found 544.2634.

(*2S,4R*)-Benzyl 2-((*S*)-1-cyano-3-phenylpropylcarbamoyl)-4-hydroxypyrrolidine-1-carboxylate (**26**) was synthesized according to general procedure from **25** (75 mg, 0.144 mmol) and n-Bu_4NF (1M solution in THF, 0.22 mL) in anh. THF (1.5 mL). After column chromatography yield was 55 mg (94.3%) as a syrup. $[\alpha]_D^{26.0}$ = -40.2 (c = 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (m, 1H), 7.71 (s, 1H), 7.43 – 6.94 (m, 11H), 5.10 (m, 2H), 4.66 (m, 1H), 4.37 (m, 2H), 3.90 – 3.15 (m, 3H), 2.67 (m, 2H), 2.39 – 1.59 (m, 5H). IR (film) ν_{max} 413.7, 425.1, 698.9, 748.4, 999.2, 1030.3, 1082.6, 1123.1, 1172.5, 1279.9, 1356.6, 1421.0, 1498.0, 1533.1, 1680.9, 2927.3, 3030.6, 3301.3 cm^{-1}. HRMS (TOF) (m/z): $M+Na^+$ calcd for $C_{23}H_{25}N_3O_4Na$ 430.1743, found 430.1725.

(*2S,4R*)-Benzyl 4-(tert-butyldimethylsilyloxy)-2-((*S*)-1-(2-(tert-butyldimethylsilyloxy)phenylamino)-1-oxo-4-phenylbutan-2-ylcarbamoyl)pyrrolidine-1-carboxylate (**28**) was obtained following general procedure from **17a** (162 mg, 0.3 mmol) and **27** [27] (48 mg, 0.36 mmol) affording 145 mg (73.7%) **28** as syrup. $[\alpha]_D^{25.4}$ = -6.3 (c = 0.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (s, 1H), 7.41 – 7.09 (m, 11H), 7.02 – 6.88 (m, 2H), 6.88 – 6.76 (m, 1H), 5.16 (s, 2H), 4.44 (d, *J* = 36.7, 3H), 3.53 (s, 2H), 2.79 – 1.83 (m, 9H), 1.26 (s, 6H), 1.00 (d, *J* = 4.9, 9H), 0.89 – 0.78 (m, 11H), 0.26 (d, *J* = 7.1, 6H), 0.12 – 0.01 (m, 6H). IR (film) ν_{max} 414.8, 418.8, 423.7, 431.7, 447.8, 668.4, 749.9, 836.8, 1114.4, 1259.0, 1355.4, 1418.0, 1455.6, 1533.3, 1600.7, 1683.6, 2342.2, 2360.0, 2854.8, 2926.6, 3629.4 cm^{-1}. HRMS (TOF) (m/z): $M+H^+$ calcd for $C_{41}H_{60}N_3O_6Si_2$ 746.4021, found 746.4047.

(*2S,4R*)-Benzyl 4-hydroxy-2-((*S*)-1-(2-hydroxyphenylamino)-1-oxo-4-phenylbutan-2-ylcarbamoyl)pyrrolidine-1-carboxylate (**29**) was synthesized following general procedure from **28** (52 mg, 0.07 mmol) and n-Bu_4NF (1M solution in THF, 0.21 mL) in anh. THF (1.5 mL), yield was 25 mg (66.3%). Mp 127 °C. $[\alpha]_D^{25.4}$ = -20.2 (c = 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.25 (s, 1H), 8.99 (s, 1H), 7.40 – 6.69 (m, 17H), 5.19 – 4.90 (m, 2H), 4.42 (m, 4H), 3.69 – 3.47 (m, 2H), 3.40 (s, 1H), 2.99 – 2.83 (m, 1H), 2.68 (m, 4H), 2.20 (m, 4H), 1.95 (m, 1H). IR (film) ν_{max} 411.9, 423.7, 428.3, 435.6, 443.9, 455.2, 697.9, 750.5, 1126.7, 1173.7, 1357.1, 1455.6, 1497.8, 1538.1, 1599.9, 1668.1, 2926.5, 3287.3 cm^{-1}. HRMS (TOF) (m/z): $M+Na^+$ calcd for $C_{29}H_{31}N_3O_6Na$ 540.2111, found 540.2124.

(*2S,4R*)-Benzyl 4-(tert-butyldimethylsilyloxy)-2-((*S*)-1-(2-(tert-butyldimethylsilyloxy)ethylamino)-1-oxo-4-phenylbutan-2-ylcarbamoyl)pyrrolidine-1-carboxylate (**31**) was obtained following general procedure from **17a** (162 mg, 0.3 mmol) and **30** [27] (58 mg, 0.33 mmol) yielding 146 mg (69.8%) **31** as syrup. $[\alpha]_D^{25.4}$ = -16.2 (c = 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (m, 12H), 6.55 (s, 1H), 5.14 (m, 2H), 4.41 (m, 3H), 4.13 (q, J = 7.1, 1H), 3.69 (s, 3H), 3.44 (m, 3H), 2.67 (m, 2H), 2.60 – 2.32 (m, 2H), 2.24 (m, 2H), 2.06 (m, 3H), 1.04 – 0.71 (m, 18H), 0.18 – 0.02 (m, 12H). IR (film) ν_{max} 416.0, 423.8, 428.1, 431.4, 435.6, 446.7, 455.0, 462.8, 473.3, 836.4, 1022.9, 1115.1, 1171.1, 1256.9, 1356.6, 1420.0, 1545.5, 1649.2, 2348.8 cm^{-1}. HRMS (TOF) (m/z): $M+H^+$ calcd for $C_{37}H_{60}N_3O_6Si_2$ 698.4021, found 698.4016.

(*2S,4R*)-Benzyl 4-hydroxy-2-((*S*)-1-(2-hydroxyethylamino)-1-oxo-4-phenylbutan-2-ylcarbamoyl)pyrrolidine-1-carboxylate (**32**) was synthesized following general procedure by deprotection of **31** (131 mg, 0.188 mmol) with n-Bu_4NF (1M solution in THF, 0.56 mL) in anh. THF (2.5 mL), yield was 77 mg (87.1%). Mp 156 °C. $[\alpha]_D^{25.3}$ = -36.6 (c = 0.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 – 6.99 (m, 12H), 5.20 – 5.00 (m, 2H), 4.61 – 4.31 (m, 3H), 3.49 (s, 2H), 3.36 (d, *J* = 4.7, 2H), 3.28 (d, *J* = 4.8, 1H), 3.19 – 3.03 (m, 5H), 2.77 – 2.57 (m, 2H), 2.48 (s, 1H), 2.35 (s, 1H), 2.25 (s, 1H), 2.15 – 1.83 (m, 3H). IR (film) ν_{max} 415.0, 429.2, 699.3, 747.8, 1123.8, 1171.5, 1356.7, 1419.3, 1539.2, 1666.8, 2875.4, 2961.0, 3285.7 cm^{-1}. HRMS (TOF) (m/z): $M+H^+$ calcd for $C_{25}H_{32}N_3O_6$ 470.2291, found 470.2273.

(*2S,4R*)-Benzyl 4-(tert-butyldimethylsilyloxy)-2-((*S*)-1-(2,2-diethoxyethylamino)-1-oxo-4-phenylbutan-2-ylcarbamoyl)pyrrolidine-1-carboxylate (**34**) was obtained following general procedure from **17a** (162 mg, 0.3 mmol) and **33** (48 mg, 0.36 mmol). It isolated 145 mg (73.7%) **34**. Mp 93 °C. $[\alpha]_D^{25.3}$ = -28.7 (c = 0.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (m, 12H), 5.24 – 4.98 (m, 2H), 4.64 – 4.28 (m, 4H), 3.71 (q, *J* = 7.0, 4H), 3.58 – 3.29 (m, 4H), 2.80 – 2.43 (m, 2H), 2.34 – 2.14 (m, 2H), 2.05 (m, 4H), 1.23 (m, 6H), 0.85 (s, 9H), 0.041 & 0.059 (2×s, 6H). IR (film) ν_{max} 413.1, 417.7, 421.2, 429.9, 437.0, 451.7, 457.0, 463.0, 668.4, 698.0, 752.2, 1126.1, 1356.9, 1423.2, 1540.1, 1669.4, 2342.8, 2360.0, 2926.9, 3304.4 cm^{-1}. HRMS (TOF) (m/z): $M+Na^+$ calcd for $C_{35}H_{53}N_3O_7SiNa$ 678.3550, found 678.3564.

(*2S,4R*)-benzyl 2-((*S*)-1-(2,2-diethoxyethylamino)-1-oxo-4-phenylbutan-2-ylcarbamoyl)-4-hydroxypyrrolidine-1-carboxylate (**35**) was obtained by deprotection of **34** (122 mg, 0.181 mmol) with n-Bu_4NF (1M solution in THF, 0.55 mL) in anh. THF (2.5 mL), yield was 93 mg (95.1%). Mp 117 °C. $[\alpha]_D^{25.0}$ = -32.8 (c = 0.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (m, 12H), 5.25 – 4.97 (m, 2H), 4.63 – 4.28 (m, 4H), 3.72 (q, *J* = 7.0, 4H), 3.57 – 3.27 (m, 4H), 2.80 – 2.43 (m, 2H), 2.33 – 2.14 (m, 2H), 2.05 (m, 4H), 1.23 (m, 6H). IR (film) ν_{max} 413.2, 417.5, 421.0, 430.9, 437.6, 451.5, 457.3, 463.6, 668.6, 698.1, 752.8, 1126.4, 1355.9, 1424.2, 1540.9, 1669.3, 2343.0, 2360.0, 2926.4, 3304.7 cm^{-1}. HRMS (TOF) (m/z): $M+H^+$ calcd for $C_{29}H_{40}N_3O_7$542.2866, found 542.2867.

(*2S,4R*)-Benzyl 4-(tert-butyldiphenylsilyloxy)-2-((*S*)-1-(2-ethoxy-2-oxoethylamino)-1-oxo-4-phenylbutan-2-ylcarbamoyl)pyrrolidine-1-carboxylate (**38**) was obtained by general method from **17b** (332 mg, 0.5 mmol) and glycine ethyl ester hydrochloride **37** (84 mg. 0.6 mmol). Column chromatographic purification yielded 305 mg (81.0%) of product **38** as syrup. $[\alpha]_D^{25.3}$ = -2.2 (c = 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 – 6.95 (m, 20H), 6.68 (s, 1H), 6.54 (s, 1H), 6.41 (s, 1H), 5.29 (s, 1H), 5.11 (m, 2H), 4.49 (m, 2H), 4.41 – 3.98 (m, 4H), 3.96 – 3.62 (m, 2H), 3.52 (m, 1H), 3.43 – 3.29 (m, 1H), 3.24 – 3.10 (m, 1H), 2.81 – 2.42 (m, 2H), 2.38 – 1.71 (m, 5H), 1.25 (m, 3H), 1.03 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.53, 169.76, 136.25, 135.58, 135.57, 129.98, 129.94, 128.57, 128.53, 128.51, 128.12, 127.85, 127.80, 127.68, 126.15, 99.98, 71.69, 71.34, 67.43, 60.00, 41.26, 38.29, 32.94, 31.96, 26.83, 26.79, 19.07, 19.04, 14.15. IR (film) ν_{max} 424.2,435.0, 611.8, 665.3, 700.2, 740.8, 821.3, 899.2, 1021.2, 1111.5, 1197.6, 1353.2, 1426.6, 1454.5, 1497.3, 1538.0, 1651.7, 1747.1, 2349.0, 2856.8, 2930.6, 3296.2 cm^{-1}. HRMS (TOF) (m/z): $M+H^+$ calcd for $C_{43}H_{52}N_3O_7Si$ 750.3575, found 750.3604.

2-((*S*)-2-((*2S,4R*)-1-(Benzyloxycarbonyl)-4-(tert-butyldiphenylsilyloxy)pyrrolidine-2-carboxamido)-4-phenylbutanamido)acetic acid (**39**) was obtained using procedure similar to synthesis **17a** from 224 mg of **38** and 0.358 mL of 1N LiOH aq. in 3 mL THF:MeOH = 1:1 mixture affording 0.206 g (96%) product **39** which was pure for using in the next step. $[\alpha]_D^{24.0}$ = -3.2 (c = 1, CH_3OH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 – 6.55 (m, 22H), 5.05 (m, 4H), 4.50 (m, 2H), 4.35 (m, 1H), 3.98 (m, 1H), 3.66 (m, 2H), 3.46 (m, 2H), 2.61 (m, 2H), 2.03 (m, 4H), 1.26 (m, 1H), 1.01 (2×s, 9H). IR (film) ν_{max} 416.3, 597.1, 697.2, 1081.2, 1123.3, 1174.7, 1211.2, 1356.7, 1424.6, 1497.3, 1547.8, 1649.9, 2096.5, 3453.1 cm^{-1}. HRMS (TOF) (m/z): $M+H^+$ calcd for $C_{41}H_{48}N_3O_7Si$ 722.3262, found 722.3279.

(*R*)-Benzyl 2-(phenyl(tosyl)carbamoyl)pyrrolidine-1-carboxylate (**42**) (model reaction) was obtained from Cbz-protected proline (**40**) and *N*-phenyl-4-toluensulfonamide (**41**) [29] using DCC as a condensing agent in presence of DMAP, according to general procedure. DCC (155 mg, 0.75 mmol) was added to the solution of 4-methyl-N-phenylbenzenesulfonamide (124 mg, 0.5 mmol), Cbz-proline (125 mg, 0.5 mmol) and DMAP (0.6 mg, 0.005 mmol) in anh. CH_2Cl_2 (5 mL) and reaction mixture was stirred for 22 h. The reaction mixture was filtered, mixed with 0.1 N HCl (5 mL), filtered again, organic phase was separated, water solution was extracted with CH_2Cl_2 (3×1 mL), organic phases were combined, dried over Na_2SO_4, filtered and concentrated in vacuo. A residue was purified by column chromatography on SiO_2 (20 g), using EtOAc/Hexanes = 5/95, 10/90, 20/80, 30/70 and 40/60 (v/v) as eluant affording 192 mg of **42** (80.3%). Mp 163 °C. $[\alpha]_D$ = 73.8 (c = 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.49 (3H, s, CH_3), 3.49 (2H, m, CH_2), 3.58 (2H, m, CH_2), 3.98 (1H, m, CH), 4.18 (1H, m, CH), 4.70 (1H, d, J = 11.7 Hz, CH), 5.01 (2H, m, CH_2), 7.1-7.5 (10H, m, PhH). IR (film) ν_{max} 419.4, 433.0, 448.1, 459.2, 468.4, 548.0, 570.2, 651.3, 677.7, 696.3, 752.1, 814.9, 982.9, 1027.8, 1086.4, 1118.8, 1172.5, 1250.1, 1356.4, 1415.5, 1451.9, 1488.7, 1541.9, 1594.8, 1712.6, 2341.5, 2360.5, 2928.3 cm^{-1}. HRMS (TOF) (m/z): M+H+ calcd for $C_{26}H_{27}N_2O_5S$ 501.1460, found 501.1458.

Biological Evaluation

Effects of compounds on parasite development were determined by the flow cytometry [35]. *P. falciparum* parasites (*D6* clone or Chloroquine resistant *W2* strain) were synchronized with 5% sorbitol and cultured at 1% parasitemia and 2% hematocrit under the atmosphere of 3% O_2, 6% CO_2, and 91% N_2 in RPMI-1640 medium supplemented with 10% human serum. Inhibitors were added from DMSO stocks (maximum concentration of DMSO equal to 0.1%). After 48 h, the culture medium was removed and replaced with 1% formaldehyde in PBS pH 7.4, and cells were fixed for 48 h at room temperature. Fixed parasites were transferred into 0.1% Triton-X-100 in PBS containing 1 nM YOYO-1 dye (Molecular Probes). Parasitemia was determined from dot plots (forward scatter vs fluorescence acquired on FACSort flow cytometer) with the help of CellQuest software (Beckton Dickinson). IC_{50} values for growth inhibition were determined from plots of percent control parasitemia over inhibitor concentration with the help of GraphPad Prism software.

All the compounds were simultaneously tested for cytotoxicity on VERO (monkey kidney fibroblast) cells by the neutral red assay [36]. IC_{50} values for each compound were computed from the growth inhibition curve.

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Supporting Information

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cbdv.2017xxxxx

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