Available online at www.sciencedirect.com
ScienceDirect
European Journal of Medicinal Chemistry 44 (2009) 1363–1368
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Preliminary communication

Synthesis and antimalarial activity of hydroxyethylpiperazine derivatives

Wilson Cunico [a,*], Claudia R.B. Gomes [a], Marcele Moreth [a], Diogo P. Manhanini [a], Isabela H. Figueiredo [b], Carmen Penido [b], Maria G.M.O. Henriques [b], Fernando P. Varotti [c], Antoniana U. Krettli [c]

[a] *Fundação Oswaldo Cruz, Instituto de Tecnologia em Fármacos, Farmanguinhos R. Sizenando Nabuco, 100, Manguinhos, 21041-250 Rio de Janeiro, RJ, Brazil*
[b] *Instituto de Tecnologia em Fármacos, Farmanguinhos, Departamento de Farmacologia Aplicada, Rio de Janeiro, RJ, Brazil*
[c] *Centro de Pesquisas René Rachou, Fiocruz e Departamento de Parasitologia (UFMG), Belo Horizonte, MG, Brazil*

Received 16 February 2008; received in revised form 18 April 2008; accepted 18 April 2008
Available online 29 April 2008

Abstract

The antimalarial activity of hydroxyethylpiperazine derivatives, synthesized from the reaction of (2*S*,3*S*)Boc-phenylalanine epoxide with benzylpiperazines in good yields (76–96%), has been evaluated *in vitro* against the *Plasmodium falciparum* W2 clone (chloroquine resistant). The results show that some compounds have moderate activity against this parasite and none of the active compounds showed cytotoxicity at high concentration (100 μg/ml).

Keywords: Hydroxyethylpiperazine; Malaria; Piperazine

1. Introduction

Malaria accounts for more than a million deaths each year, of which over 80% occur in tropical Africa, where malaria is the leading cause of mortality in children under five years of age [1]. In Brazil it is estimated that 500,000 cases occur annually, mainly in the Amazon Rain Forest. *Plasmodium falciparum* is the most dangerous form of the four malarial parasites that infect humans. Despite the importance of this disease, there has been little economic incentive for the development of new drug-based antimalarial therapies. There is a growing need for effective drugs with new mechanism of action, due to the high rate of mutation of the parasite, which leads to the development of resistance. One of the critical stages of the life cycle of the parasite during human infection is the degradation of hemoglobin which provides nutrients for its growth and maturation [2]. A family of aspartic proteases, known as the plasmepsins [3,4], appear to be involved in the initial steps of the degradation pathway and therefore make for an attractive antimalarial drug target [5]. There are four aspartyl proteases present in the food vacuole of *P. falciparum*, plasmepsins I, II, IV and Histo-Aspartic-Protease (HAP). The most attractive target from this group of enzymes is the plasmepsin II [6]. Recent studies have shown that HIV protease inhibitors can inhibit the plasmepsin II *in vitro* [7,8] and *in vivo* [9] in the food vacuole at pharmacologically relevant concentrations.

A secondary alcohol is usually the structural element of choice to inhibit aspartic protease. This element mimics the tetrahedral intermediate during peptide bond cleavage by aspartic proteases [10]. It has been successfully used to develop potent inhibitors of these enzymes, for example dihydroxyethylene- [11] and hydroxyethylamine-based [12] molecules (Fig. 1). In this work, we report the synthesis and *in vitro* activity of novel hydroxyethylpiperazine-based compounds against *P. falciparum*. The clone W2 of *P. falciparum* was used for the test [13], as described in our previous work [14,15], and activity compared to that of lopinavir and

* Corresponding author. Tel.: +55 21 3977 2463.
E-mail address: wjcunico@far.fiocruz.br (W. Cunico).

0223-5234/$ - see front matter © 2008 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2008.04.009

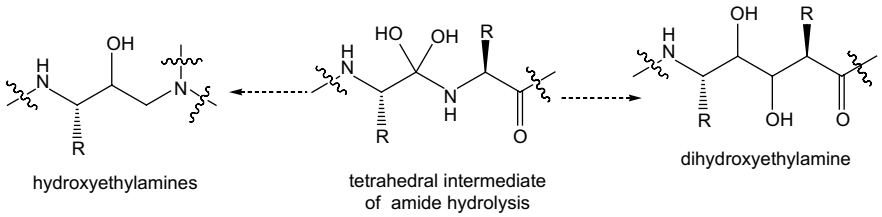


Fig. 1. Molecules developed for the synthesis of aspartyl protease inhibitors.

saquinavir (Fig. 2), used as in parallel in each test. Lopinavir is the HIV protease inhibitor which has the best activity against *P. falciparum* [8].

2. Chemistry

The hydroxyethylamine transition-state mimicking fragment was prepared by selective ring-opening of the (2*S*,3*S*)Boc-phenylalanine epoxide **1** (prepared according to the method of Beaulieu) [16] with secondary amines (piperazine derivatives) [17]. The epoxide stirred with four different piperazines **2a–d** in isopropanol at reflux temperature affords compounds **3a–d** in good yields (Scheme 1, route a). Table 1 shows the antimalarial activities of compounds **3a–d** expressed as 50% inhibitory concentration (IC_{50}) of *P. falciparum* growth.

In this first screening, we observed that only compound **3d** (entry 4) shows moderate activity. This compound has a CH_2 space linker between piperazine and the phenyl group. We next synthesized more compounds with CH_2 space linkers. A mixture of epoxide **1** and 4-benzylpiperazines **2e–j** in refluxing isopropanol for 16 h gave the hydroxyethylpiperazine derivatives **3e–j** in good yields (Scheme 1, route b). Compound **3k** was prepared by a reduction reaction from compound **3h**. The nitro group of compound **3h** was reduced with H_2 and Pd/C (10%) using ethanol as the solvent, in quantitative yield. Both analytical and spectral data (^1H and ^{13}C NMR) of all compounds are in full agreement with the proposed structures. 2D-NMR techniques (HMBQ, HMQC and COSY) helped us to assign the correct signals of compounds. As an example, the ^1H NMR for compound **3j** exhibits a double doublet for H1a at 2.96 ppm ($J = 14.0$ and $^2J = 4.8$ Hz) and multiplet for H1b at 2.87 ppm. Protons H2 and H3 are shown as a broad signal at 3.80 and 3.62 ppm, respectively, and proton H4 appears together with the piperazine signals at 2.45 ppm. The signal for the methylene protons of benzylpiperazine showed a singlet at 3.41 ppm. The ^{13}C NMR spectra exhibit broad signals at 53.2 and 52.9 ppm for the piperazine carbons (pp). Other general ^{13}C NMR signals occur about δ 36.4 (C1), 54.5 (C2), 68.0 (C3) and 61.0 (C4) ppm.

Commercially unavailable precursor piperazines required to prepare compounds **3e–j** of Scheme 1 were synthesized according to a previously reported method that is outlined in Scheme 2 [18]. The mixture of 1 equiv of piperazine with 1 equiv of piperazine dihydrochloride hydrate in absolute ethanol (65 °C) gave a solution containing 1 equiv of piperazine monohydrochloride (Scheme 2, route a). Slow addition of 1 equiv of benzyl chloride to the above warm solution produced 1 equiv of corresponding monoalkylated piperazine and 1 equiv of precipitated piperazine dihydrochloride [19]. The unavailable benzyl chlorides were synthesized from corresponding arenealdehydes (Scheme 2, route b). The reaction of aldehyde with sodium borohydride gives the corresponding alcohol that is reacted with hydrochloric acid (37%) to afford the benzyl chlorides [20].

3. Pharmacological evaluation

Parasites were cultured with human erythrocytes (blood group O+) at 5% hematocrit in RPMI 1640 supplemented with 10% human plasma as previously described [21]. Test compounds were solubilized in ethanol prior to *in vitro* tests. The antiparasitic effects of the molecules were measured by the [^{3}H]-hypoxanthine incorporation assay [22]. Briefly, trophozoite stages in sorbitol-synchronized blood [23] were cultured at 2% parasitaemia and 2.5% hematocrit, in the presence of the test compounds (at various concentrations), diluted with culture medium (RPMI 1640) without hypoxanthine; a chloroquine control (as a reference antimalarial drug) was used in each experiment. Inhibition of parasite growth was evaluated through the levels of [^{3}H]-hypoxanthine incorporation plotted to generate dose–response curves. The half-maximal inhibitory response (IC_{50}) compared with parasite growth in the drug-free controls was estimated by curve fitting using a software program [Microcal, Origin Software, Inc. (Northampton, MA, USA)].

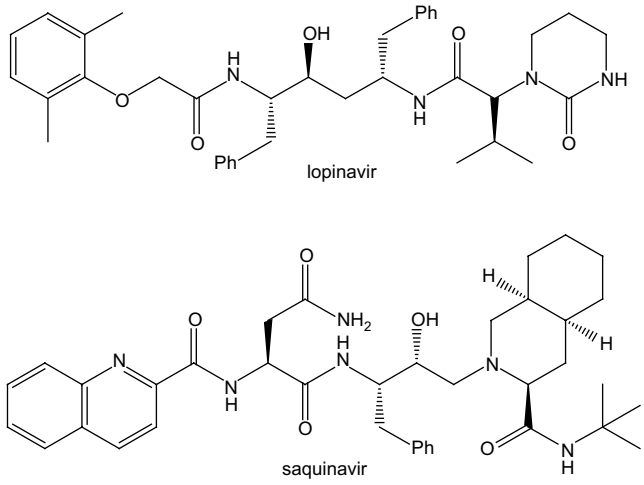
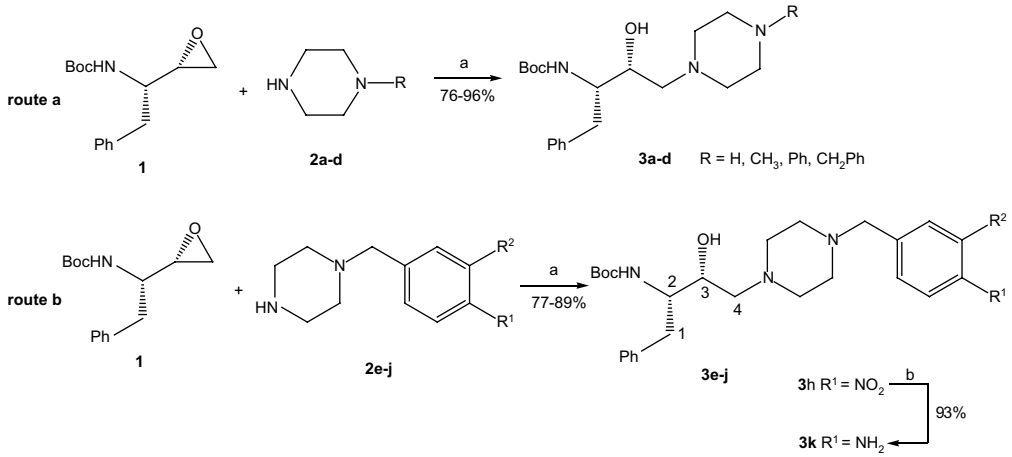


Fig. 2. Structure of HIV protease inhibitors lopinavir and saquinavir.



Scheme 1. Reagents and conditions: (a) IPA, reflux, 16 h; (b) H_2, Pd/C 10%, r.t., 16 h.

4. Cytotoxicity assay

Cytotoxicity was determined in the murine monocyte/macrophage cell lineage J774, using the method previously described [24]. The cells were seeded in a flat bottom 96-well plate (2×10^6 cells/well) cultured for 1 h (5% CO_2 at 37 °C) in RPMI 1640 enriched with 10% bovine fetal serum, 2 mM L-glutamine, 25 μg/ml gentamicin, pH 7.4. Adherent cells were cultured in the presence of different concentrations of the compounds (0.1–100 μg/ml) for 20 h, when MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) solution (5 mg/ml; 22.5 μl/well) was added to the culture for 4 h. Supernatant was discharged and DMSO (150 μl/well) was added for solubilization of formazan crystals. The absorbance was read at 540 nm.

5. Results and discussion

The most marked effect is observed when a methylene space linker was incorporated in piperazine residue. As the IC_{50} values in Table 1 indicate, the new series of hydroxyethylpiperazine derivatives has moderate antimalarial activity. It is evident that compound **3c** which did not have the methylene space linker has no activity at the maximum concentration (50 μg/ml) used (entry 3, Table 1). The same results were observed for compounds **3a** (R = H) and **3b** (R = Me). The compound **3d** ($R = CH_2Ph$) shows moderate antimalarial activity (entry 4). Introduction of a halogen atom into the para position of the benzylpiperazine group resulted in an increase in potency (entries 5 and 6). Compound **3j**, with the piperazine ring substituted with a piperonyl group, has the best antimalarial activity with an IC_{50} of 5.1 μg/ml, being as active as saquinavir mesylate used as the standard (entry 10). The hydroxyethylpiperazine **3h**, that has a strong electron withdrawing group, showed poor antimalarial activity ($IC_{50} = 23.5$ μg/ml).

6. Conclusion

In summary, the hydroxyethylpiperazines **3a–k** could be synthesized easily with good yields. Some compounds showed moderate antimalarial activity and also were not cytotoxic to host cells at high concentration (100 μg/ml). The CH_2 space linker was found to be critical for activity, since compounds **3a–c** had been without antimalarial activity. Further work with these hydroxyethylamine derivatives is now undertaken to increase the antimalarial activity, especially, with compounds that have the piperonyl piperazine moiety.

7. Experimental section

Unless otherwise indicated, all common reagents and solvents were used as obtained from commercial suppliers without further purification. All melting points were determined on a Büchi Melting Point B-545 and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Bruker DRX 400 spectrometer (^1H at 400.14 MHz and ^{13}C at 100.61 MHz) and on a Bruker Avance 500 spectrometer (^1H at 500.13 MHz and ^{13}C at 125.75 MHz) in $CDCl_3$ containing TMS as an internal standard. Microanalyses were performed on Perkin–Elmer Model 2400 instrument and all values were within ±0.4% of the theoretical values. LC–MS analyses were performed on a LC–MS micromass ZMD using methanol (100%) as mobile phase with flux of 0.3 ml/min.

7.1. General procedure

Benzylpiperazines **2a–j** (1.5 mmol) and epoxide **1** (1.6 mmol) were dissolved in 2-propanol (10 ml) and refluxed overnight (100 °C). The solvent was removed by evaporation and the crude product was purified by crystallization in methanol/water (7:3) to give hydroxyethylpiperazine derivatives **3a–j**.

7.1.1. Compound *3a*

M.p. 145–147 °C. ^1H NMR (500 MHz, $CDCl_3$): 7.32–7.21 (m, 5H, Ph); 4.58 (d, 1H, NH, $J = 9.0$ Hz); 3.82 (br, 1H, H2); 3.65 (br, 1H, H3); 2.97 (dd, 1H, H1a, $J = 13.5$ Hz, $^2J = 4.5$ Hz); 2.86 (m, 1H, H1b); 2.73 (br, 2H, pp); 2.49 (br, 8H, H4 and pp); 1.35 (s, 9H, Boc). ^{13}C NMR (125 MHz, $CDCl_3$): 155.6 (CO); 137.7; 129.6; 129.5; 128.5; 128.4;

Table 1
Yields, *in vitro* antimalarial activity against *P. falciparum* W2 clone and cytotoxicity of compounds **3a–k**

Entry	Compound	R^1	R^2	Yield[a] (%)	IC_{50} μg/ml[b] (μM)	Cytotoxicity[c] (% of cell viability)			
						0.1 μg/ml	1 μg/ml	10 μg/ml	100 μg/ml
1	**3a** [25]	–	–	89	>50	100	100	100	65
2	**3b**	–	–	76	>50	94	100	100	100
3	**3c** [25]	–	–	80	>50	100	95	94	58
4	**3d**	H	H	96	14.9 (35.0)	95	100	100	100
5	**3e**	F	H	77	7.5 (16.9)	100	100	99	90
6	**3f**	Cl	H	85	7.7 (16.7)	100	100	100	88
7	**3g**	OMe	H	89	6.2 (13.2)	100	100	100	85
8	**3h**	NO_2	H	85	23.5 (50.0)	100	100	98	81
9	**3i**	OMe	OMe	68	7.0 (14.1)	100	100	100	83
10	**3j**	$-OCH_2O-$		86	5.1 (10.8)	100	98	100	82
11	**3k**	NH_2	H	93	15.8 (34.8)	100	98	98	85
Chloroquine					0.14				
Saquinavir					7.0 (9.1)				
Lopinavir					1.7 (2.7)				

[a] Yields of isolated compounds.
[b] IC_{50} represents concentration inhibitory dose of the parasite growth in relation to control cultures with no drugs.
[c] Percentage of J774 cell viability 24 h after incubation with the compounds determined by the MTT method.

126.3; 79.4 (Boc); 67.9 (C3); 60.9 (C4); 54.3 (C2); 53.8 (br, pp); 36.2 (C1); 28.3 (Boc). LC–MS (*m/z*) (%): 350.14 ($M^+ + 1$, 100); 249 (37); 182 (100); 96 (15). Anal. Calcd for $C_{19}H_{31}N_3O_3$: C, 65.30; H, 8.94; N, 12.02. Found: C, 65.64; H, 9.14; N, 12.22.

7.1.2. Compound *3b*

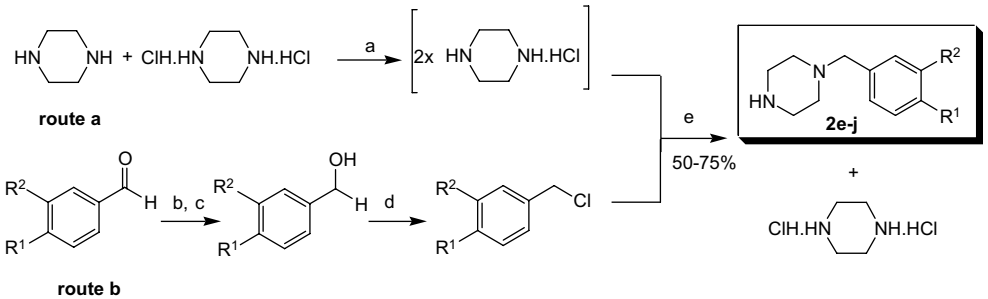
M.p. 114–115 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 7.31–7.20 (m, 5H, Ph); 4.58 (d, 1H, NH, $J = 9.0$ Hz); 3.81 (br, 1H, H2); 3.60 (br, 1H, H3); 2.97 (dd, 1H, H1a, $J = 14.0$ Hz, $^2J = 4.0$ Hz); 2.87 (m, 1H, H1b); 2.71 (br, 2H, pp); 2.53 (s, 3H, CH_3); 2.48 (br, 8H, H4 and pp); 1.34 (s, 9H, Boc). ^{13}C NMR (125 MHz, $CDCl_3$): 155.6 (CO); 137.8; 129.6; 129.5; 128.3; 128.2; 126.3; 79.3 (Boc); 67.9 (C3); 60.8 (C4); 55.0 (C2); 54.4 and 52.8 (pp); 45.8 (CH_3); 36.3 (C1); 28.2 (Boc). LC–MS (*m/z*) (%): 364.34 ($M^+ + 1$, 100); 264 (2). Anal. Calcd for $C_{20}H_{33}N_3O_3$: C, 66.08; H, 9.15; N, 11.56. Found: C, 65.92; H, 9.21; N, 11.60.

7.1.3. Compound *3c*

M.p. 183–185 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 7.32–7.22 (m, 10H, Ph); 4.64 (d, 1H, NH, $J = 9.0$ Hz); 3.85 (br, 1H, H2); 3.68 (br, 1H, H3); 2.99 (dd, 1H, H1a, $J = 14.0$ Hz, $^2J = 4.5$ Hz); 2.90 (m, 1H, H1b); 2.78 (br, 2H, pp); 2.50 (br, 8H, H4 and pp); 1.35 (s, 9H, Boc). ^{13}C NMR (125 MHz, $CDCl_3$): 155.6 (CO); 137.7; 130.4; 129.6; 128.4; 128.3; 127.2; 126.3; 79.4 (Boc); 68.2 (C3); 61.0 (C4); 54.4 (C2); 52.9 and 46.9 (pp); 36.3 (C1); 28.3 (Boc). LC–MS (*m/z*) (%): 364.34 ($M^+ + 1$, 100); 264 (2). Anal. Calcd for $C_{25}H_{35}N_3O_3$: C, 70.56; H, 8.29; N, 9.87. Found: C, 70.54; H, 8.30; N, 9.84.

7.1.4. Compound *3d*

M.p. 96–98 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 7.34–7.19 (m, 10H, Ph); 4.57 (d, 1H, NH, $J = 8.5$ Hz); 3.86 (br, 1H, H2); 3.64 (br, 1H, H3); 3.52 (s, 2H, CH_2); 2.96 (dd, 1H, H1a, $J = 14.0$ Hz, $^2J = 4.0$ Hz); 2.87 (m, 1H, H1b); 2.70 (br, 2H, pp); 2.48 (br, 8H, H4 and pp); 1.34 (s, 9H, Boc). ^{13}C NMR (125 MHz, $CDCl_3$): 155.6 (CO); 137.8; 129.6; 129.2; 128.4; 128.3; 127.2; 126.3; 79.3 (Boc); 67.9 (C3); 62.9 (CH_2); 61.0 (C4); 54.3 (C2); 52.9 and 52.8 (pp); 36.2 (C1); 28.2 (Boc). LC–MS (*m/z*) (%): 440.27 ($M^+ + 1$, 100); 384 (6); 267 (4); 182 (98); 177 (47); 91 (97). Anal. Calcd for



Scheme 2. Reagents and conditions: (a) EtOH, 78 °C, 1 h; (b) $NaBH_4$ (0.5 equiv), EtOH, 0 °C, r.t., 18 h; (c) HCl 15%; (d) $SOCl_2$, py, r.t., 4 h; (e) EtOH, 78 °C, 2 h.

$C_{26}H_{37}N_3O_3$: C, 71.04; H, 8.48; N, 9.56. Found: C, 71.44; H, 8.59; N, 9.86.

*7.1.5. Compound **3e***

M.p. 115–117 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 7.30–7.20 (m, 7H, Ph); 7.00 (d, 2H, Ph, $J = 8.5$ Hz); 4.57 (d, 1H, NH, $J = 7.6$ Hz); 3.80 (br, 1H, H2); 3.72 (br, 1H, H3); 3.50 (s, 2H, CH_2); 2.98 (dd, 1H, H1a, $J = 14.0$ Hz, $^2J = 4.4$ Hz); 2.87 (m, 1H, H1b); 2.78 (br, 2H, pp); 2.55 (br, 8H, H4 and pp); 1.34 (s, 9H, Boc). ^{13}C NMR (100 MHz, $CDCl_3$): 162.2 (d, $J_{CF} = 245.1$ Hz); 155.6 (CO); 137.7; 130.7 (d, $^3J_{CF} = 7.6$ Hz); 129.6; 128.4; 126.4; 115.2 (d, $^2J_{CF} = 20.9$ Hz); 79.5 (Boc); 68.0 (C3); 61.8 (CH_2); 61.4 (C4); 54.4 (C2); 53.3 and 52.1 (pp); 36.3 (C1); 28.3 (Boc). LC–MS (*m/z*) (%): 458.58 ($M^+ + 1$, 100); 364 (22); 303 (15). Anal. Calcd for $C_{26}H_{36}FN_3O_3$: C, 68.25; H, 7.93; N, 9.18. Found: C, 68.41; H, 8.20; N, 9.32.

*7.1.6. Compound **3f***

M.p. 128–129 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 7.30–7.19 (m, 9H, Ph); 4.57 (d, 1H, NH, $J = 9.0$ Hz); 3.81 (br, 1H, H2); 3.64 (br, 1H, H3); 3.47 (s, 2H, CH_2); 2.96 (dd, 1H, H1a, $J = 14.0$ Hz, $^2J = 4.0$ Hz); 2.88 (m, 1H, H1b); 2.69 (br, 2H, pp); 2.48 (br, 8H, H4 and pp); 1.33 (s, 9H, Boc). ^{13}C NMR (125 MHz, $CDCl_3$): 155.6 (CO); 137.8; 136.5; 132.8; 130.4; 129.6; 128.4; 128.3; 126.3; 79.3 (Boc); 67.9 (C3); 62.1 (CH_2); 61.0 (C4); 54.3 (C2); 52.9 and 52.8 (pp); 36.3 (C1); 28.3 (Boc). LC–MS (*m/z*) (%): 474.58 ($M^+ + 1$, 100); 364 (63); 264 (14); 214 (10). Anal. Calcd for $C_{26}H_{36}ClN_3O_3$: C, 65.88; H, 7.65; N, 8.86. Found: C, 65.90; H, 7.66; N, 8.86.

*7.1.7. Compound **3g***

M.p. 110–112 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 7.28 (d, 2H, Ph, $J = 7.5$ Hz); 7.25–7.21 (m, 5H, Ph); 6.87 (d, 2H, Ph, $J = 8.5$ Hz); 4.57 (d, 1H, NH, $J = 9.5$ Hz); 3.82 (br, 1H, H2); 3.81 (s, 3H, OCH_3); 3.66 (br, 1H, H3); 3.49 (s, 2H, CH_2); 2.97 (dd, 1H, H1a, $J = 13.9$ Hz, $^2J = 4.5$ Hz); 2.88 (m, 1H, H1b); 2.74 (br, 2H, pp); 2.51 (br, 8H, H4 and pp); 1.34 (s, 9H, Boc). ^{13}C NMR (100 MHz, $CDCl_3$): 159.0; 155.7 (CO); 138.1; 130.6; 129.8; 128.5; 126.5; 113.8; 79.5 (Boc); 68.2 (C3); 62.5 (CH_2); 61.2 (C4); 55.5 (OCH_3); 54.6 (C2); 53.4 and 53.0 (pp); 36.5 (C1); 28.5 (Boc). LC–MS (*m/z*) (%): 470.67 ($M^+ + 1$, 100); 364 (15); 264 (15). Anal. Calcd for $C_{27}H_{39}N_3O4$: C, 69.05; H, 8.37; N, 8.95. Found: C, 69.39; H, 8.23; N, 8.88.

*7.1.8. Compound **3h***

M.p. 148–150 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 8.17 (d, 2H, Ph, $J = 9.0$ Hz); 7.50 (d, 2H, Ph, $J = 8.5$ Hz); 7.30–7.19 (m, 5H, Ph); 4.56 (d, 1H, NH, $J = 9.0$ Hz); 3.81 (br, 1H, H2); 3.60 (br, 1H, H3); 3.59 (s, 2H, CH_2); 2.95 (dd, 1H, H1a, $J = 14.0$ Hz, $^2J = 4.5$ Hz); 2.87 (m, 1H, H1b); 2.67 (br, 2H, pp); 2.45 (br, 8H, H4 and pp); 1.34 (s, 9H, Boc). ^{13}C NMR (125 MHz, $CDCl_3$): 155.6 (CO); 147.1; 146.2; 137.8; 129.6; 129.5; 128.3; 126.3; 123.5; 79.3 (Boc); 67.9 (C3); 62.0 (CH_2); 60.8 (C4); 54.3 (C2); 53.3 and 53.1 (pp); 36.3 (C1); 28.3 (Boc). LC–MS (*m/z*) (%): 485.33 ($M^+ + 1$, 96); 364 (100); 301 (31); 264 (15). Anal. Calcd for $C_{26}H_{36}N_4O_5$: C, 64.44; H, 7.49; N, 11.56. Found: C, 64.32; H, 7.51; N, 11.68.

*7.1.9. Compound **3i***

M.p. 125–126 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 7.30–7.20 (m, 7H, Ph); 6.81 (d, 2H, Ph, $J = 8.5$ Hz); 4.57 (d, 1H, NH, $J = 10.0$ Hz); 3.89 (s, 3H, OCH_3); 3.87 (s, 3H, OCH_3); 3.81 (br, 1H, 2H); 3.69 (br, 1H, H3); 3.55 (s, 2H, CH_2); 2.97 (dd, 1H, H1a, $J = 13.5$ Hz, $^2J = 4.0$ Hz); 2.87 (m, 1H, H1b); 2.73 (br, 2H, pp); 2.51 (br, 8H, H4 and pp); 1.34 (s, 9H, Boc). ^{13}C NMR (125 MHz, $CDCl_3$): 155.6 (CO); 148.8; 148.2; 137.7; 129.6; 129.1; 128.4; 126.3; 121.4; 110.7; 79.4 (Boc); 67.9 (C3); 62.9 (CH_2); 61.0 (C4); 55.9 (OCH_3); 55.8 (OCH_3); 54.4 (C2); 53.3 and 52.7 (pp); 36.3 (C1); 28.3 (Boc). LC–MS (*m/z*) (%): 500.43 ($M^+ + 1$, 100); 400 (3); 151 (3). Anal. Calcd for $C_{28}H_{41}N_3O_5$: C, 67.31; H, 8.27; N, 8.41. Found: C, 66.98; H, 8.48; N, 8.23.

*7.1.10. Compound **3j***

M.p. 103–105 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 7.30–7.18 (m, 5H, Ph); 6.84 (s, 1H, Ph); 6.74 (s, 2H, Ph); 5.94 (s, 2H, OCH_2O); 4.57 (d, 1H, NH, $J = 8.4$ Hz); 3.80 (br, 1H, H2); 3.62 (br, 1H, H3); 3.41 (s, 2H, CH_2); 2.96 (dd, 1H, H1a, $J = 14.0$ Hz, $^2J = 4.8$ Hz); 2.87 (m, 1H, H1b); 2.67 (m, 2H, pp); 2.45 (br, 8H, H4 and pp); 1.34 (s, 9H, Boc). ^{13}C NMR (100 MHz, $CDCl_3$): 155.6 (CO); 147.7; 146.7; 137.9; 131.9; 129.6; 128.3; 126.3; 122.2; 109.5; 107.9; 100.9 (OCH_2O); 79.3 (Boc); 68.0 (C3); 62.7 (CH_2); 61.0 (C4); 54.5 (C2); 53.2 and 52.9 (pp); 36.4 (C1); 28.3 (Boc). LC–MS (*m/z*) (%): 484.49 ($M^+ + 1$, 100); 384 (4). Anal. Calcd for $C_{27}H_{37}N_3O_5$: C, 67.06; H, 7.71; N, 8.69. Found: C, 67.06; H, 7.72; N, 8.69.

*7.1.11. Compound **3k***

To a stirred solution of compound **3h** (280 mg, 0.57 mmol) in absolute ethanol (15 ml) under a blanket of nitrogen was added 10% palladium on activated charcoal (5 mg). The reaction was placed under a hydrogen atmosphere and stirred overnight. The reaction mixture was filtered through Celite, and the solvent was removed under reduced pressure to yield **3k** as a brown oil. The residue was purified by chromatography on silica, eluting with 3:1 hexane/EtOAc. **3k**: m.p. 139–142 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 7.30–7.20 (m, 5H, Ph); 7.08 (d, 2H, Ph, $J = 8.0$ Hz); 6.96 (d, 2H, Ph, $J = 8.0$ Hz); 4.62 (d, 1H, NH, $J = 9.5$ Hz); 3.81 (m, 1H, H2); 3.61 (br, 1H, H3); 3.42 (s, 2H, CH_2); 2.96 (dd, 1H, H1a, $J = 15.0$ Hz, $^2J = 4.0$ Hz); 2.86 (m, 1H, H1b); 2.69 (br, 2H, pp); 2.45 (br, 8H, H4 and pp); 1.34 (s, 9H, Boc). ^{13}C NMR (125 MHz, $CDCl_3$): 155.5 (CO); 145.5; 137.7; 130.5; 129.7; 129.5; 128.3; 128.2; 126.3; 126.2; 115.2; 79.2 (Boc); 67.9 (C3); 62.3 (CH_2); 61.7 (C4); 54.3 (C2); 52.6 and 52.3 (pp); 36.2 (C1); 28.3 (Boc). IR (KBr): 3450 (NH_2); 3354 (NH); 2970 and 2924 (CH_2 and CH_3); 1706 (CO). Anal. Calcd for $C_{26}H_{38}N_4O_3$: C, 68.69; H, 8.43; N, 12.32. Found: C, 68.42; H, 8.63; N, 12.15.

Acknowledgements

The authors thank Farmanguinhos, FAPERJ and CNPq for the financial support of the research.

References

[1] (a) W. Cunico, S.A. Carvalho, C.R.B. Gomes, G.H. Marques, Rev. Bras. Farm. 89 (2008) 49;
(b) B.M. Greenwood, K. Bojang, C.J. Whitty, G.A. Targett, Lancet 365 (2005) 1487.
[2] G.H. Coombs, D.E. Goldberg, M. Klemba, C. Berry, J. Kay, J.C. Mottram, Trends Parasitol. 17 (2001) 532.
[3] C. Boss, S. Richard-Bildstein, T. Weller, W. Fischli, S. Meyer, C. Binkert, Curr. Med. Chem. 10 (2003) 883.
[4] H. Gutiérrez-de-Terán, M. Nervall, K. Ersmark, P. Liu, L.K. Janka, B. Dunn, A. Hallberg, J. Aqvist, Biochemistry 45 (2006) 10529.
[5] C. Dash, A. Kulkarni, B. Dunn, M. Rao, Crit. Rev. Biochem. Mol. Biol. 38 (2003) 89.
[6] K. Ersmark, B. Samuelsson, A. Hallberg, Med. Res. Rev. 26 (2006) 626.
[7] T.S. Skinner-Adams, J.S. McCarthy, D.L. Gardiner, P.M. Hilton, K.T. Andrews, J. Infect. Dis. 190 (2004) 1998.
[8] S. Parikh, J. Gut, E. Istvan, D.E. Goldberg, D.V. Havlir, P.J. Rosenthal, Antimicrob. Agents Chemother. 49 (2005) 2983.
[9] K.T. Andrews, D.P. Fairlie, P.K. Madala, J. Ray, D.M. Wyatt, P.M. Hilton, L.A. Melville, L. Beattie, D.L. Gardioner, R.C. Reid, M.J. Stoermer, T. Skinner-Adams, C. Berry, J.S. McCarthy, Antimicrob. Agents Chemother. 50 (2006) 639.
[10] R.C. Ogden, C.W. Flexner (Eds.), Protease Inhibitors in AIDS Therapy, Marcel Dekker Inc., New York, 2001.
[11] (a) K. Ersmark, I. Feierberg, S. Bjelic, E. Hamelink, F. Hackett, M.J. Blackman, J. Hultén, B. Samuelsson, J. Aqvist, A. Hallberg, J. Med. Chem. 47 (2004) 110;
(b) K. Ersmark, M. Nervall, H. Gutiérrez-de-Terán, E. Hamelink, L.K. Janka, J.C. Clemente, B.M. Dunn, A. Gogoll, B. Samuelsson, J. Aqvist, A. Hallberg, Bioorg. Med. Chem. 14 (2006) 2197.
[12] (a) D. Noteberg, E. Hamelink, J. Hultén, M. Wahlgren, L. Vrang, B. Samuelsson, A. Hallberg, J. Med. Chem. 46 (2003) 734;
(b) D. Muthas, D. Noteberg, Y.A. Sabnis, E. Hamelink, L. Vrang, B. Samuelsson, A. Karlén, A. Hallberg, Bioorg. Med. Chem. 13 (2005) 5371;
(c) T.S. Haque, A.G. Skillman, C.E. Lee, H. Habashita, I.Y. Gluzman, T.J. Ewing, D.E. Goldberg, I.D. Kuntz, J.A. Ellman, J. Med. Chem. 42 (1999) 1428.
[13] M.G. Zalis, L. Pang, M.S. Silveira, W.K. Milhous, D.F. Wirth, Am. J. Trop. Med. Hyg. 58 (1998) 630.
[14] W. Cunico, C.A. Cechinel, H.G. Bonacorso, M.A.P. Martins, N. Zanatta, M.V.N. de Souza, I.O. Freitas, R.P.P. Soares, A.U. Krettli, Bioorg. Med. Chem. Lett. 16 (2006) 649.
[15] W. Cunico, M.L.G. Ferreira, T.G. Ferreira, C. Penido, M.G.M.O. Henriques, L.G. Krettli, F.P. Varotti, A.U. Krettli, Lett. Drug Des. Discov. 5 (2008) 178.
[16] P.L. Beaulieu, D. Wernic, J.-S. Duceppe, Y. Guindon, Tetrahedron Lett. 36 (1995) 3317.
[17] (a) H. Tamamura, Y. Koh, S. Ueda, Y. Sasaki, T. Yamasaki, M. Aoki, K. Maeda, Y. Watai, H. Arikuni, A. Otaka, H. Mitsuya, N. Fujii, J. Med. Chem. 46 (2003) 1764;
(b) P.L. Beaulieu, P.C. Anderson, D.R. Cameron, G. Croteau, V. Gorys, C. Grand-Maitre, D. Lamarre, F. Liard, W. Paris, L. Plamondon, F. Soucy, D. Thibeault, D. Wernic, C. Yoakin, J. Med. Chem. 43 (2000) 1094.
[18] A.S. Mehanna, J.Y. Kim, Bioorg. Med. Chem. 13 (2005) 4323.
[19] J.C. Craig, R.J. Young, Org. Syntheses Coll. 5 (1973) 88.
[20] S.J. Howell, N. Spencer, D. Philp, Tetrahedron 57 (2001) 4945.
[21] W. Trager, J.B. Jensen, Science 193 (1976) 673.
[22] R.E. Desjardins, C.J. Canfield, J.D. Haynes, J.D. Chulay, Antimicrob. Agents Chemother. 16 (1979) 710.
[23] C. Lambros, J.P. Vanderberg, J. Parasitol. 65 (1979) 418.
[24] M. Ferrari, M.C. Fornasiero, A.M. Isetta, J. Immunol. Methods 131 (1990) 165.
[25] R.M. McConnell, A.W. Green, C.J. Trana, M.S. McConnell, J.F. Lindley, K. Sayyar, W.E. Godwin, S.E. Hatfield, Med. Chem. 2 (2006) 27.