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Synthesis and Antimalarial Activity of Cinnamic Acid Derivatives

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ABSTRACT

A peptide coupling reaction between 1-(R)-phenylethylalanine and cinnamic acid derivatives has been successfully employed for the synthesis of a set of small molecules. The antimalarial activity of these derivative molecules is reported. The compounds have been evaluated against chloroquine-sensitive (3D7) and chloroquine-resistant (W2) strains of P. falciparum as well as their cytotoxic activity against HUVEC cells. For 3D7, the most active molecule was compound 8 with IC50 of 23.6 nM/mL comparable to that of chloroquine (18.5 nM/mL). The products were characterized by IR, NMR and MS analysis.

Keywords: Peptide coupling; Cinnamic acid; Reagent-based diversity; Antimalarial; *P. falciparum*

INTRODUCTION

This year's OMS [1] report shows that after an unprecedented period of success in global malaria control, progress has stalled. In 2016, there were an estimated 216 million cases of malaria, an increase of about 5 million cases over 2015. Deaths reached 445 000, a similar number to the previous year.

Malaria-related mortality followed the same trend, i.e., a decline from 2010 to 2014, and then an increase in 2015 and 2016. According to this report, it is in the WHO African region that the increase in cases of malaria and associated deaths was the most significant. The African region still accounts for some 90% of malaria cases and related deaths worldwide. Fifteen countries, all in sub-Saharan Africa but one, account for 80% of the global burden of malaria.

One of the biggest challenges facing malaria chemotherapy is the rapid emergence of resistance to existing antimalarial drugs [2]. Chloroquine was replaced as first line therapy by the sulfonamide antimalarials and later on, artemisinin combination therapy (ACT), following the development of widespread resistance against the drug by Plasmodium falciparum [3]. This challenge underscores the need for the continued search for new antimalarials.

The routes research [4-11] are being pursued for the discovery of new antimalarial with less side effects, a faster onset of action and a better rate of response.

In the process of searching for new small molecules interacting with the strain P. *falciparum*, we have identified the compound derivatives of cinnamic acid as a promising scaffold. In this paper, we describe the synthesis of new derivatives with as potential antimalarial properties.

EXPERIMENTAL SECTION

General Procedures

Commercial reagents were used without purification. Prior to use, CH3CN, DMSO and Methanol were dried using a pure solvent drying system over aluminum oxide under an argon atmosphere. All anhydrous reactions were carried out under nitrogen atmosphere. Analytical thin layer chromatography was performed on SDS silica gel 60F254 aluminium plates (0.2 mm layer) and was revealed by UV light and/or by phosphomolybdic acid. All flash chromatography separations were performed with SDS silica gel 60. Melting points (mp) were determined on a Tottoli apparatus and were uncorrected. Infrared (IR) spectra were obtained as neat films and were recorded on Bruker Vector 22 spectrophotometer. 1H and 13C spectra were recorded in CD3OD or CDCl3 either on a Bruker Avance 300 or 600 MHz and 75 or 150 MHz, respectively. Chemicals shifts (δ) are reported in ppm relative to TMS for 1H and 13C NMR spectra. The following abbreviations are used to indicate the multiplicities: s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet), quint (quintet) and m (multiplet). GC/MS conditions: Analyses were performed using a 5890 gas chromatogram connected to a G 1019 A mass spectrometer (both from Hewlett Packard) operating in the electro spray ionization mode (ESI).

Experimental Details of Synthesis of Cinnamic Acid Derivatives

General procedure for the coupling réaction of 1-(*r*)-phenylethylamine 1 with cinnamic acid derivatives 2.

A solution of cinnamic acid derivatives (1 equiv), α-amino ester hydrochloride (2 equiv), EDCI.HCl (2.3 equiv), DIEA (4 equiv), and HOBt.H_2O (2.3 equiv) in MeCN was stirred for 48h at rt and under Ar. The reaction mixture was then diluted with AcOEt (80 ml) and washed with HCl 10% (80 ml). The organique layer was washed with saturated aqueous solution of $NaHCO_3$ (80 ml), H_2O (80 mL), and brine (80 ml). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (Cyclohexane/AcET).

(*E*)-3-(4-fluorophenyl) -*N*-((R)-1-phenylethyl) acrylamide (4)

Following the general procedure, cinnamic acid derivatives 3 (R_1=H, R_2=F, R_3=H) (168 mg, 1.012 mmol) reacted with phenylethylamine (248.534 mg, 2.054 mmol), EDCI.HCl (440.48 mg, 2.297 mmol), DIEA (0.71 mL, 4.04 mmol), and HOBt.H_2O (326.8 mg, 2.41 mmol) in MeCN (4 mL). Purification of the residue on silica gel (Cyclohexane/AcEt 5:5).

(*E*)-3-(3,4-dimethoxyphenyl) -*N*-((*R*)-1-phenylethyl) acrylamide (5).

Following the general procedure, cinnamic acid derivatives 3 (R_1=OCH_3, R_2=OCH_3, R_3=H) (210.5 mg, 1.012 mmol) reacted with phenylethylamine (248.534 mg, 2.054 mmol), EDCI.HCl (440.48 mg, 2.297 mmol), DIEA (0.71 mL, 4.04 mmol), and HOBt.H_2O (326.8 mg, 2.41 mmol) in MeCN (4 mL). Purification of the residue on silica gel (Cyclohexane/AcEt 5:5).

(*E*)-3-(3-hydroxy-4,5-dimethoxyphenyl) -*N*-((R)-1-phenylethyl) acrylamide (6)

Following the general procedure, cinnamic acid derivatives 3 (R_1=OH, R_2=OCH3, R_3=OCH3) (224.04 mg, 1.012 mmol) reacted with phenylethylamine (248.534 mg, 2.054 mmol), EDCI.HCl (440.48 mg, 2.297 mmol), DIEA (0.71 mL, 4.04 mmol), and HOBt.H_2O (326.8 mg, 2.41 mmol) in MeCN (4 mL). Purification of the residue on silica gel (Cyclohexane/AcEt 5:5).

(*E*)-3-(3-hydroxy-4-methoxyphenyl) -*N*-((R)-1-phenylethyl) acrylamide (7)

Following the general procedure, cinnamic acid derivatives 3 (R_1=OH, R_2=OCH3, R_3=H) (194.38 mg, 1.012 mmol) reacted with phenylethylamine (248.534 mg, 2.054 mmol), EDCI.HCl (440.48 mg, 2.297 mmol), DIEA (0.71 mL, 4.04 mmol), and HOBt.H_2O (326.8 mg, 2.41 mmol) in MeCN (4 mL). Purification of the residue on silica gel (Cyclohexane/AcEt 5:5).

(*E)*-3-(3,4-dihydroxyphenyl) -*N*-((R)-1-phenylethyl) acrylamide (8).

Following the general procedure, cinnamic acid derivatives 3 (R_1=OH, R_2=OH, R_3=H) (182.9 mg, 1.012 mmol) reacted with phenylethylamine (248.534 mg, 2.054 mmol), EDCI.HCl (440.48 mg, 2.297 mmol), DIEA (0.71 mL, 4.04 mmol), and HOBt.H_2O (326.8 mg, 2.41 mmol) in MeCN (4 mL). Purification of the residue on silica gel (Cyclohexane/AcEt 5:5).

Antiplasmodial Assay

The antimalarial activity of extracts/compounds was evaluated against *P. falciparum* 3D7 and *P. falciparum* W2 strains, using the fluorescence-based SYBR Green I assay approach in 96-well microplates [12] with some modifications. Positive control wells for each assay contained no inhibitor while negative controls contained Chloroquine (CQ). The CQ molecule was provided from World Wide Antimalarial Resistance Network (wwarn Network). Experiments were run in duplicate with both test and control drugs employed at varying concentrations. Stock solutions (extracts) were prepared in dimethyl-sulfoxide (DMSO) and diluted with culture medium to give a maximum DMSO concentration of 0.5 % in a final well volume of 200 µL containing 1 % parasitemia and 2.5 % haematocrit. Compounds and negative control [Chloroquine (CQ)] were prepared by two-fold dilution, in a dose-titration range of 0.098-100 µg/mL, to obtain 11 concentrations each, in duplicate. The concentrations used for CQ were between 0.5 and 1000 nM. After 48 h incubation, the plates were subjected to 3 freeze thaw cycles to achieve complete hemolysis. The parasite lysis suspension was diluted 1:5 in SYBR Green I lysis buffer (10 mM NaCl, 1 mM Tris HCl pH8, 2.5 mM EDTA pH 8, 0.05 % SDS, 0.01 mg/mL proteinase K and 10X SYBR Green I). Incorporation of SYBR Green I in parasite DNA amplification was measured using the Master epRealplex cycler® (Eppendorf, France) according the following program to increase the SYBR green incorporation: 90°C for 1 min, decrease in temperature from 90°C to 10°C for 5 min with reading the fluorescence 10°C for 1 min and a new reading at 10°C for 2 min. The IC_{50} was calculated by nonlinear regression using icestimator website 1.2 versions: http://www.antimalarial-icestimator.net/MethodIntro.htm.

Cytotoxicity on HUVEC

HUVEC cells were cultured in Gibco™ RPMI 1640 medium (Life technologies, France) complemented with 10% Fetal Bovine Serum and 1 mM L-glutamine (Sigma-Aldrich, France) and incubated in 5% CO_2 at 37°C. The cytotoxicity of extracts was evaluated using the SYBR Green I assay as previously described. HUVEC were seeded

in a 96-well plate at 100,000 cells/well and incubated for 24 h to adhere. After discarding the old medium, the cells were incubated in the medium containing eight concentrations (0.78-100 µg/mL) of each extract in duplicate. After 48h incubation, cells were visualized using an inverted microscope to check their morphology or the cell viability. The medium was subsequently removed and replaced by lysis buffer without SYBR Green I and the plates were subjected to 3 freeze-thaw cycles. The cell lysis suspension was diluted 1:2 in SYBR Green I lysis buffer. The incorporation of SYBR Green I in cell DNA and the IC_{50} analysis were obtained as previously.

RESULT AND DISCUSSION

This study describes the synthesis of molecules derived from cinnamic acid (Table 1) as well as the examination of their antiplasmodic activities (Table 2). The key compound 3 has been synthesized through a one-step process according to the Figure 1. Coupling of 1-(*R*) - phenylethylamine 1 with cinnamic acid 2 using HOBT.H_2O, EDCI.HCl/DIPEA [13,14] in acetonitrile provided the derivatives of cinnamic acids 3 with correct yields (figure 1, Table 1). The synthesized molecules and their yields are consigned in Table 1.

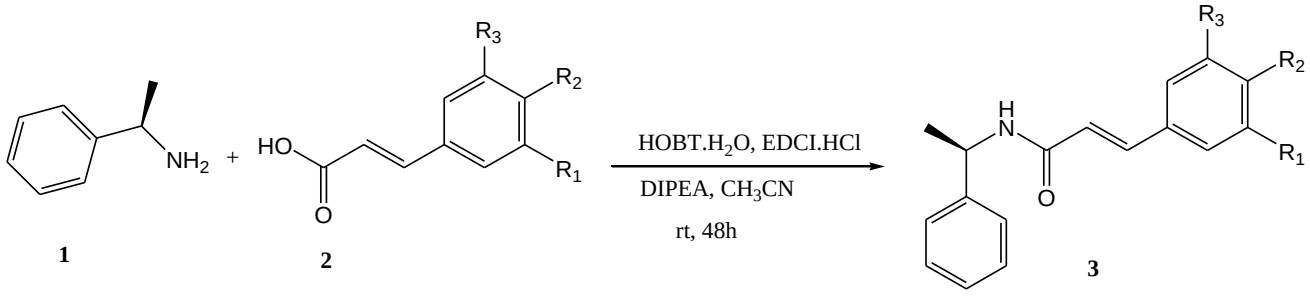

Figure 1. Synthesis of compound 3 acid cinnamic derivatives

Table 1. The products of the synthesis of 1 recorded

Compound	R	Yields %
4	R2=H, R2=F, R3=H	86%
5	R1=H, R2=OMe, R3=OMe	91%
6	R1=OH, R2=OMe, R3=OMe	70%
7	R1=OH, R2=OMe, R3=H	59%
8	R1=H, R2=OH R3=OH	78%

Table 2. The antimalarial activity and cytotoxicity of compounds of derivatives cinnamic acids recorded

	***Plasmodium falciparum* 3D7 strain**	***Plasmodium falciparum* W2 strain**	**HUVEC cells**

Compounds	IC_{50} ± SD (nM/mL)	IC_{50} ± SD (nM/mL)	CC_{50} nM/mL ± SD
4	>100	>100	>100
5	>100	>100	>100
6	49.7 ± 11	>100	>100
7	47.6 ± 10	>100	>100
8	23.6 ± 0.5	>100	>100
CQ	18.5± 0.65	>100	>100

CQ: Chloroquine

Characteristics of Synthetic Molecules

(*E*)-3-(4-fluorophenyl) -*N*-((R)-1-phenylethyl) acrylamide (4), yield: 0.2354g (86%)

IR cm^{-1} : 16.5 (CO) ; MS (ESI) m/z : 270.10 [M+1] ; RMN (MeOD, 600 Mhz) : 1.5 (d, J=6.9 Hz, CH_3) ; 3.25 (s, NH) ; 5.3 (m, 1H, CH) ; 6.5 (d, J=15.5 Hz, CH) ; 7-7.39 (m, 9H, 9×CH) ; 7.50 (d, 15.7 Hz, CH). ^{13}CRMN (MeOD, 150 Mhz): δ 21.78 CH_3 ; 49.11 CH ; 115.96 CH ; 116.10 CH ; 120.52 CH ; 126.40 2×CH ; 127.60 CH ; 127.60 2×CH ; 128.86 2×CH ; 129.65 CH ; 129.70 CH ; 140.17 CH ; 143.14 C ; 162.81(d, J=248.85 Hz, C) ; 165.47 CO.

(*E*)-3-(3,4-dimethoxyphenyl) -*N*-((*R*)-1-phenylethyl) acrylamide (5), yield: 0.2866g (91%)

IR cm^{-1} : 168.5 (CO) ; MS (ESI) m/z : 312.2 [M+1] ; RMN (MeOD, 600 Mhz) : 1.5 (d, J=6.9 Hz, CH_3) ; 3.25 (s, NH) ; 3.72 (s, 9H, 3CH3) ; 5.3 (m, 1H, CH) ; 6.5 (d, J=15.48 Hz, CH) ; 7-7.39 (m, 8H, 8×CH) ; 7.50 (d, 15.48 Hz, CH). ^{13}CRMN (MeOD, 150 Mhz) : δ 21.83 CH_3 ; 48 CH ; 55.87 CH_3 ; 55.99 CH_3 ; 109.70 CH ; 111.13 CH ; 118.74 CH ; 122 CH ; 126.35 2×CH ; 127.43 CH ; 127.9 C ; 128.75 2×CH ; 141.07 CH ; 143.31 C ; 149.13 C ; 150.58 C ; 165.47 CO.

(*E*)-3-(3-hydroxy-4,5-dimethoxyphenyl)-*N*-((R)-1-phenylethyl) acrylamide (6),yield:0.2343g (70%)

IR cm^{-1} : 168.5 (CO) ; MS (ESI) m/z : 328.10 [M+1] ; RMN (MeOD, 600 Mhz) : 1.5 (m, 3H, CH_3) ; 3.25 (s, NH) ; 3.9 (s, 6H, $2CH_3$) ; 5.3 (m, 1H, CH) ; 6.8 (d, J=15 Hz, CH) ; 7-7.39 (m, 7H, 7×CH) ; 7.50 (d, 15 Hz, CH). ^{13}CRMN (MeOD, 150 Mhz) : δ 21.84 CH_3 ; 49.03 CH, 56.42 2×CH_3 ; 104.84 CH ; 118.72 CH ; 126.39 2×CH ; 126.44 C ; 127.57 2×CH ; 128.85 2×CH ; 136.67 C ; 141.59 CH ; 143.24 C ; 147.29 C ; 165.47 CO.

(*E*)-3-(3-hydroxy-4-methoxyphenyl) -*N*-((R)-1-phenylethyl) acrylamide (7), yield: 0.1763g (59%)

IR cm^{-1} : 168 (CO) ; MS (ESI) m/z : 298.10 [M+1] ; RMN (MeOD, 600 Mhz) : 1.5 (m, 3H, CH_3) ; 3.3 (s, NH) ; 3.9 (s, 3H, CH_3) ; 5.2 (m, 1H, CH) ; 6.8 (d, J=15 Hz, CH) ; 7-7.39 (m, 8H, 8×CH) ; 7.50 (d, 15 Hz, CH). ^{13}CRMN (MeOD, 150 Mhz) : δ 21.84 CH_3 ; 49.02 CH, 55.98 CH_3 ; 109 CH ; 114.87 CH ; 118.31 CH ; 122.2 CH ; 126.37 CH ; 127.42 C ; 127.50 CH ; 128.80 2×CH ; 141.39 CH ; 143.29 C ; 146 C, 147.54 C ; 165.47 CO.

(*E)*-3-(3,4-dihydroxyphenyl) -*N*-((R)-1-phenylethyl) acrylamide (8) , yield : 0.1971g (78%).

IR cm^{-1} : 168.5 (CO) ; MS (ESI) m/z : 284.1 [M+1] ; RMN (MeOD, 600 Mhz) : 1.5 (d, J=6.9 Hz, CH_3) ; 3.25 (s, NH) ; 5.3 (m, 1H, CH) ; 6.5 (d, J=15.48 Hz, CH) ; 7-7.39 (m, 8H, 8×CH) ; 7.50 (d, 15.48 Hz, CH). ^{13}CRMN

(MeOD, 150 Mhz) : δ 21.83 CH_3 ; 48 CH ; 55.87 CH_3 ; 55.99 CH_3 ; 109.70 CH ; 111.13 CH ; 118.74 CH ; 122 CH ; 126.35 2×CH ; 127.43 CH ; 127.9 C ; 128.75 2×CH ; 141.07 CH ; 143.31 C ; 149.13 C ; 150.58 C ; 165.47 CO.

The best yield was obtained with molecule 5 (91%) followed by molecules 4 (86%) and 8 (78%) while molecules 6 and 7 give a modest yield (70%).

An article Kanaani et al. [15] has reported that cinnamic acid derivatives are known inhibitors of monocarboxylate transport across plasma and mitochondrial membranes. In this study all the derivatives were found to inhibit the growth of Plasmodium falciparum intra-erythrocyte in culture, which correlates with their hydrophobic character.

This prompted us to evaluate the antiplasmodic of our molecules against P. falciparum-resistant chloroquine-sensitive 3D7 and chloroquine W2 and their cytotoxic activity against HUVEC cells (Table 2). The compounds presented activities in the nanomolar range against one of the stem parasites. Their cytotoxicity against HUVEC is >100 nM.

The five synthetic products and negative control [Chloroquine (CQ)] were prepared by two-fold dilution, in a dose-titration range of 0.098-100 µg/mL, to obtain 11 concentrations each, and all of ,then showed inactivity against W2 (IC50 >100). Compound 8 (IC_{50}=23.6 ± 0.5 nM/mL) exhibited the highest activity against 3D7 followed by 6 (49.7 ± 11 nM/mL) and 7(47.6 ± 10 nM/mL). Compounds 6 and 7 have similar activity against 3D7. Compounds 4 and 5 showed inactivity against 3D7. Interestingly, activity against 3D7 is observed with compounds having a free phenolic hydroxyl. In addition, the presence of a second hydroxyl function seems to play a role in the activity.

In view of the observed results we believe that in the activity structure relationship the partie acid cinnamic has played a crucial role in the activity of these derived molecules. This result confirms the observed results [16-18] on the bioactivitie role of cinnamic acid.

CONCLUSION

In this study, we have prepared a small set of new nitrogen heterocycles displaying scaffold using a flexible chemistry. Five new derivatives cinnamic acid were prepared in good yield. The antimalarial activity of these compounds has been described. The compounds were tested against P. falciparum 3D7 strains and W2. The best result is obtained with compound 8 against 3D7.

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