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Synthesis and Study of Antioxidant Activities of *trans*-(-)-Clovamide Derivatives

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Abstract: A peptide coupling reaction between L-phenylalanine (L-DOPA) and cinnamic acids derivatives has been successfully employed for the synthesis of a set of small molecules derived from trans (-) clovamide. The antioxidant activity of these derivative molecules is reported. The antioxydant and antiradical activity were determined using DPPH (2-2-Diphenyl picrylhydrazyl) radical. The molecules which exhibit interesting antioxidant activity were: compound 5 (IC_{50} =3.46±0.034µg/ml); compound 6 (IC_{50} =3.04±0.01 µg/ml); compound 7 (IC_{50} =4.23±0.02 µg/ml); compound 8 (IC_{50} =5.1±0.061 µg/ml); compound 9 (IC_{50} =1.55±0.17 µg/ml); compound 10 (IC_{50} =6.02±0.07 µg/ml) and compound 13 (IC_{50} =2.49±0.06 µg/ml). These molecules contain polyphenols wich are generally very good antioxidants. Thus, this study showed that compound 9 with an IC_{50} of 1.55 µg/ml has antioxidant activity close to that of quercetin (IC_{50} = 1.20µg/ml), a well known antioxidant compound.

Keywords: Antioxidant, Antiradical, *trans*-(-)-Clovamide, DPPH

1. Introduction

Oxidative stress is involved in the development of many pathologies as a triggering factor or associated with complications. Most of the diseases induced by oxidative stress appear with age because aging decreases antioxidant defenses and increases the mitochondrial multiplication of radicals [1]. Oxidative stress is the primary cause of several diseases [2]. It is the factor potentiating the appearance of multifactorial diseases such as type 2 diabetes, Alzheimer's disease, obesity, rheumatism, atherosclerosis, cancers and cardiovascular diseases [3-4]. The concept of an antioxidant therapy, with the aim of strengthening the endogenous antioxidant defense for a more effective protection against oxidative stress, represents an important therapeutic issue of scientific and public interest. From molecules isolated from plants, it is possible to modulate their pharmacological activities by different structural modifications by hemi-synthesis or total synthesis. It is in this context that our study aimed at evaluating the antioxidant activity of nine synthetic molecules. We report the synthesis of molecules derived from *trans* (-) clovamide [5-7], which we isolated in the leaf of *Icacina olivifomis (Poiret) Raynal* and the experimental study of their antioxidant activities. *Icacina oliverfomis (Poiret) Raynal* also known as *I. senegalensis* is a traditional Senegalese medicinal plant which leaves are used in Senegal for the treatment of diabetes [8] and other diseases [4].

2. Materials and Methods

2.1. Molecules Studied

The molecules which antioxidant activity were evaluated consisted of synthetic substances derived from trans (-)

clovamide and encoded 5, 6, 7, 8, 9, 10, 11, 12 et 13.

2.2. Reagents and Solvent Used

The ethanol used was supplied by the Technical House (Dakar Senegal). The DPPH**.** was supplied by Sigma – Aldrich (Saint Quentin Fallavier, France). Quercetin was obtained from Extrasynthesis (Genay France) and ascorbic acid was supplied by Panreac (Lyon, France).

2.3. Method of Synthesis of the Molecules Studied

A synthesis of the *trans* (-) clovamide 4 derivatives has been proposed, consisting of a coupling of the *L*-DOPA 2 (2a[9]) α-Amino ester hydrochlorides with cinnamic acid derivatives 3. α-Amino ester hydrochloride 2 was obtained by the action of thionyl chloride in MeOH on the derivatives of *L*-DOPA 1 in excellent yield. Coupling of hydrochloride 2 with cinnamic acid derivatives 3 using HOBT.H_2O, EDCI.HCl / DIPEA [9, 10] in acetonitrile provided the derivatives of *trans* (-) clovamide 4 with correct yields (Figure 1). The synthesized molecules and their yields are consigned in Table 1.

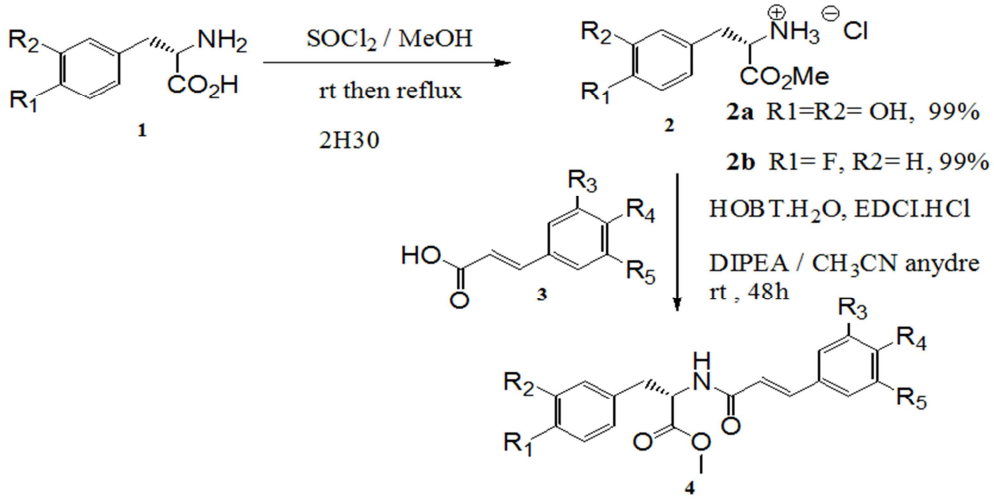

Figure 1. *Synthesis of derivatives of trans (-) clovamide 4.*

Table 1. *Yields derivatives of trans (-) clovamide 4.*

Entry	Product	R1	R2	R3	R4	R5	Yield
1	5	OH	OH	H	OH	OCH3	92%
2	6	OH	OH	OH	OCH_3	OCH_3	77%
3	7	OH	OH	OCH3	OCH_3	H	80%
4	8	OH	OH	H	F	H	90%
5	9[a]	OH	OH	H	OH	OH	80%
6	10	OH	OH	F	F	H	87%
7	11	F	H	H	F	H	96%
8	12	F	H	F	F	H	89%
9	13	F	H	OH	OH	H	91%

([a]) molecule described in the literature [6, 8]

2.4. Experimental Details of the Synthesis of Molecules

General: Commercial reagents were used without purification. Prior to use, CH_3CN 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 60F_{254} 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 ^{13}C spectra were recorded in CD3OD or $CDCl_3$ either on a Bruker Avance 300 or 500 MHz and 75 or 125 MHz, respectively. Chemicals shifts (δ) are reported in ppm relative to TMS for ^1H and ^{13}C 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), m (multiplet). The microanalysis has been done on a Perkin-Elmer 2400-CMN apparatus. 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).

4-fluorophenyl-L-alanine methyl ester hydrochloride (2b). A solution of 4-fluorophenyl-L-aniline (1g, 5.46 mmol) in methanol (24 mL) is added dropwise $SOCl_2$ (0.7 ml, 9.74 mmol) at room temperature under argon. The mixture was refluxed for 2h30, then maintained overnight at 20°C. The solution concentrated under reduced pressure and dried at the pump.

General Procedure for the Coupling Réaction of α-Amino Esters hydrochlororides 2 with Cinnamique Acid derivatives 3.

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), a 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 (CH_2Cl_2/MeOH).

Methyl ester of N- (4'- hydroxy- 3'-methoxy-*trans*-cinnamoy1)-3-(3, 4-dihydroxyphenyl)-L-alanine (5). Following the general procedure, cinnamic acid derivatives 3 (R_3=OCH_3, R_4=OH, R_5=H) (194.38 mg, 1.01 mmol) reacted with L-alanine methyl ester hydrochloride 2a (499.08 mg, 2.02 mmol), EDCI.HCl (444.74 mg, 2.32 mmol), DIEA (0.71 mL, 4.04 mmol), and HOBt.H_2O (313.48 mg, 2.32 mmol) in MeCN (4 mL). Purification of the residue on silica gel (CH_2Cl_2/MeOH 9:1).

Methyl ester of N-(3'-hydroxy-4', 5'-dimethoxy-*trans*-cinnamoy1)-3-(3,4-dihydroxyphenyl)-*L*-alanine (6). Following the general procedure, cinnamic acid derivatives 3 (R_3=OH, R_4=OCH_3, R_5=OCH_3) (226.68 mg, 1.01 mmol) reacted with L-alanine methyl ester hydrochloride 2a (499.08 mg, 2.02 mmol), EDCI.HCl (444.74 mg, 2.32 mmol), DIEA (0.71 mL, 4.04 mmol), and HOBt.H_2O (313.48 mg, 2.32 mmol) in MeCN (4 mL). Purification of the residue on silica gel (CH_2Cl_2/MeOH 9:1).

Methyl ester of N- (3', 4'-dimethoxy-*trans*-cinnamoy1)-3-(3, 4-dihydroxyphenyl)-L-alanine (7). Following the general procedure, cinnamic acid derivatives 3 (R_3=OCH_3, R_4=OCH_3, R_5=H) (210.05 mg, 1.01 mmol) reacted with L-alanine methyl ester hydrochloride 2a (499.08 mg, 2.02 mmol), EDCI.HCl (444.74 mg, 2.32 mmol), DIEA (0.71 mL, 4.04 mmol), and HOBt.H_2O (313.48 mg, 2.32 mmol) in MeCN (4 mL). Purification of the residue on silica gel (CH_2Cl_2/MeOH 9:1).

Methyl ester of N- (4'-fluoro-*trans*-cinnamoy1)-3-(3, 4-dihydroxyphenyl)-L-alanine (8). Following the general procedure, cinnamic acid derivatives 3 (R_3=H, R_4=F, R_5=H) (168 mg, 1.01 mmol) reacted with L-alanine methyl ester hydrochloride 2a (499.08 mg, 2.02 mmol), EDCI.HCl (444.74 mg, 2.32 mmol), DIEA (0.71 mL, 4.04 mmol), and HOBt.H_2O (313.48 mg, 2.32 mmol) in MeCN (4 mL). Purification of the residue on silica gel (CH_2Cl_2/MeOH 9:1).

Methyl ester of N- (3', 4'-difluoro-*trans*-cinnamoy1)-3-(3, 4-dihydroxyphenyl)-L-alanine (10). Following the general procedure, cinnamic acid derivatives 3 (R_3=F, R_4=F, R_5=H) (226.68 mg, 1.01 mmol) reacted with L-alanine methyl ester hydrochloride 2a (499.08 mg, 2.02 mmol), EDCI.HCl (444.74 mg, 2.32 mmol), DIEA (0.71 mL, 4.04 mmol), and HOBt.H_2O (313.48 mg, 2.32 mmol) in MeCN (4 mL). Purification of the residue on silica gel (CH_2Cl_2/MeOH 9:1).

Methyl ester of N- (4'-fluoro-*trans*-cinnamoy1)-3-(4-fluorophenyl)-L-alanine (11). Following the general procedure, cinnamic acid derivatives 3 (R_3=H, R_4=F, R_5=H) (226.68 mg, 2.05 mmol) reacted with L-alanine methyl ester hydrochloride 2a (233 mg, 1 mmol), EDCI.HCl (440.491 mg, 2.30 mmol), DIEA (0.71 mL, 4.04 mmol), and HOBt.H_2O (310.77 mg, 2.30 mmol) in MeCN (4 mL). Purification of the residue on silica gel (CH_2Cl_2/AcET 9:1).

Methyl ester of N- (4'-fluoro-*trans*-cinnamoy1)-3-(3, 4-difluorophenyl)-L-alanine (12). Following the general procedure, cinnamic acid derivatives 3 (R_3=F, R_4=F, R_5=H) (377.26 mg, 2.05 mmol) reacted with L-alanine methyl ester hydrochloride 2a (233 mg, 1 mmol), EDCI.HCl (440.491 mg, 2.30 mmol), DIEA (0.71 mL, 4.04 mmol), and HOBt.H_2O (310.77 mg, 2.30 mmol) in MeCN (4 mL). Purification of the residue on silica gel (CH_2Cl_2/AcET 9:1).

Methyl ester of N- (3', 4'-dihydroxy-*trans*-cinnamoy1)-3-(4-fluorophenyl)-L-alanine (13). Following the general procedure, cinnamic acid derivatives 3 (R_3=OH, R_4=OH, R_5=H) (369.08 mg, 2.05 mmol) reacted with L-alanine methyl ester hydrochloride 2a (233 mg, 1 mmol), EDCI.HCl (440.491 mg, 2.30 mmol), DIEA (0.71 mL, 4.04 mmol), and HOBt.H_2O (310.77 mg, 2.30 mmol) in MeCN (4 mL). Purification of the residue on silica gel (CH_2Cl_2/AcET 9:1).

2.5. Measurement of Antioxidant Activity by DPPH Test

The antiradical activity of the nine synthetic derivatives of clovamide is measured by the 2,2'-diphenyl-1-picryl-hydrazyl test according to method previously described [11, 12].

Indeed, an amount of 4mg of DPPH powder was dissolved in 100ml of ethanol and the solution obtained was kept away from light for 12 h.

In each test tube containing 0.8 ml of an ethanolic solution of the extract tested at different initial concentrations varying from 1 to 10 µg/ml, the DPPH solution (3.2 ml) was added. Ascorbic acid and quercetin used as reference antioxidants were also tested at concentrations ranging from 1 to 10 µg/ml.

The absorbance was measured after 30 minutes at the spectrophotometer at 517 nm using ethanol as blank.

Three measurements of the absorbance were performed in triplicate for each concentration tested (n=3). From the inhibition percentage [11] of tested molecules, the IC_{50} (concentration of antioxidant molecule that inhibits half initial absorbance of the DPPH radical), EC_{50} (amount of antioxidant molecule needed to decrease the initial concentration of DPPH radical of 50%) were determined [13].

The EC_{50} expressed in grams of extract per mole of DPPH, was calculated according to the following formula, starting from the IC_{50}

EC_{50} = IC_{50} (µg / ml) / MDPPH**.** (mMol), MDPPH= molarity of the DPPH solution.

2.6. Statistical Analysis and Expression of Results

Statistical analysis were performed by the student test using Microsoft Excel 2007 software.

The results of the inhibition tests of the absorbance of the radical were considered to be significant when the *p* value is less than 0.05. The Statview version software (5.0) was used to determine the half inhibitory concentration of molecules

tested.

3. Results

3.1. Characteristics of Synthetic Molecules

4-fluorophenyl-L-alanine methyl ester hydrochloride (2b). Yield: 1.259 g (99%). IR cm^{-1} 3360, 1727.35 CO. ^{1}H RMN (MeOD, 500 Mhz): 3.1 (dd, J=7.4, 13.8 Hz, 1H, CH_2), 3.3 (dd, J=6.73, 13.8 Hz, 1H, CH_2), 3.8 (s, 3H, CH), 4.3 (t, J=7.1 Hz, 1H, CH), 7.1 (d, J=8.1 Hz, 2H, CH), 7.3 (d, J=8.2 Hz, 2H, CH). ^{13}C RMN (MeOD, 125 Mhz): δ 32.39 CH_2, 53.63 CH, 55.16 CH_3, 116.55 (d, J=21.30 Hz, 2xCH), 130.43 (d, J= 3.33 Hz, C), 132.35 (d, J= 7.70Hz, 2CH), 163.27 (d, J= 250 Hz, CF), 170.32 (CO).

Methyl ester of N- (4'- hydroxy- 3'-methoxy-*trans*-cinnamoy 1)-3-(3, 4-dihydroxyphenyl) L-alanine (5). Yield: 361.8 mg (92%). IR cm^{-1}: 1731.75 (CO ester); 1685.48 (CO amide). MS (ESI) m/z: 410.13 $[M+Na]^{+}$. MP=95°C. ^{1}H RMN (MeOD, 500 Mhz): 2.96 (dd, J=7.4, 13.8 Hz, 1H, CH_2), 3.09 (dd, J=6.73, 13.8 Hz, 1H, CH_2), 3.90 (s, 3H, CH), 4.70 (t, J=7.1 Hz, 1H, CH), 6.53 (d, J=15.70, CH); 6.70-7.08 (m, 6H, 6xCH), 7.47 (d, J=15.7Hz, CH). ^{13}CRMN (MeOD, 125 Mhz): δ 38.09 CH_2, 52.82 CH, 55.80 CH_3, 56.42 CH_3, 111.58 CH, 116.46 CH, 116.50 CH, 117.33 CH, 118.08 CH, 121.75 CH, 123.48 CH, 128.48 C, 129.38 C, 143.00CH, 145.32 C, 146.28 C, 149.22 C, 149.11 C, 168.98 CO, 173.89 CO. Anal. Calcd for:($C_{20}H_{21}NO_7$): C, 62.01, H, 5.46, N, 3.62, Found: C, 62.05, H, 5.50, N, 3.65.

Methyl ester of N-(3'-hydroxy-4', 5'-dimethoxy-*trans*-cinnamoy1)-3-(3,4-dihydroxyphenyl)-*L*-alanine (6). Yield: 324.40 mg (77%). IR cm^{-1}: 1733.65 (CO ester); 1681.38 (CO amide). MS (ESI) m/z: 440.14 $[M+Na]^{+}$. MP=105°C. ^{1}H RMN (MeOD, 500 Mhz): 3.10 (dd, J=7.4, 13.8 Hz, 1H, CH_2), 3.30 (dd, J=6.73, 13.8 Hz, 1H, CH_2), 3.80 (s, 3H, CH), 4.30 (t, J=7.1 Hz, 1H, CH), 6.51 (d, J=15.70, CH), 6.68-6.97 (m, $5H_{Ar}$), 7.46 (d, J=15.7Hz, CH). ^{13}C RMN (MeOD, 125 Mhz): δ 38.05 CH_2, 52.64 CH, 55.71 CH_3, 56.76 ($2xCH_3$), 106.48 (2xCH), 116.32 CH, 117.20 CH, 118.48 CH 121.58 CH, 127.09 C, 129.38 C, 138.99 CH, 143.04 C, 145.32 C, 146.28 C, 149.41 (2xC), 168.79 CO, 173.74 CO. Anal. Calcdfor: ($C_{21}H_{23}NO_8$): C, 60.43, H, 5.55, N, 3.36, Found: C, 60.45, H, 5.54, N, 3.37.

Methyl ester of N- (3', 4'-dimethoxy-*trans*-cinnamoy 1)-3-(3, 4-dihydroxyphenyl)-L-alanine (7). Yield: 324.13 mg (80%). IR cm^{-1}: 1732.25 (CO ester); 1680.47 (CO amide). MS (ESI) m/z: 424.15 $[M+Na]^{+}$. MP=95°C. ^{1}H RMN (MeOD, 500 Mhz): 2.88 (dd, J=7.4, 13.8 Hz, 1H, CH_2), 3.02 (dd, J=6.73, 13.8 Hz, 1H, CH_2), 3.70 (s, 3H, CH), 3.80 (s, 3H, CH), 4.73 (t, J= 7.1 Hz, 1H, CH), 6.52 (d, J=15.70, CH), 6.65-6.94 (m, $5H_{Ar}$), 7.45 (d, J=15.7Hz, CH). ^{13}C RMN (MeOD, 125 Mhz): δ 38.03 CH_2, 52.63 CH, 56.38 CH_3, 56.40 CH_3, 111.44 CH, 112.63 CH, 116.31 CH, 117.19 CH 118.95 CH, 121.75 CH, 121.56 CH, 129.22 C, 129.36 C, 143.42 CH, 145.29 C, 146.25 C, 150.64 C, 152.29 C, 168.68 CO, 173.71 CO. Anal. Calcd for: ($C_{21}H_{23}NO_7$): C, 62.83, H, 5.78, N, 3.49, Found: C, 62.85, H, 5.75, N, 3.45.

Methyl ester of N- (4'-fluoro-*trans*-cinnamoy 1)-3-(3, 4-dihydroxyphenyl)-L-alanine (8). Yield: 326.44 mg (90%). IR cm^{-1}: 1761.38 (CO ester); 1686.60 (CO amide). MS (ESI) m/z:382.12 $[M+Na]^{+}$. ^{1}H RMN (MeOD, 500 Mhz): 3.01 (dd, J=7.4, 13.8 Hz, 1H, CH_2), 3.20 (dd, J=6.73, 13.8 Hz, 1H, CH_2), 4.80 (t, J=7.1 Hz, 1H, CH), 6.48 (d, J=15.70, CH), 6.7-7.23 (m, $7H_{Ar}$), 7.35 (d, J=15.7Hz, CH). ^{13}C RMN (MeOD, 125 Mhz): δ 37.73 CH_2, 52.71 CH, 55.45 CH_3, 115.12 (2x CH), 116.03 (d, J=18.18 Hz, CH), 116.45 CH, 117.56 CH, 122.24 CH, 122.95 (J= 73.94, CH), 128.18 C, 131.94 (d, J=7.19 Hz, CH), 134.19 (d, J=2.9 Hz, C), 143.12 CH, 146.73 C, 148.45 C, 164.16 (d, J=242.94, CF), 169.02 CO, 173.45 CO. Anal. Calcd for: ($C_{19}H_{18}FNO_5$): C, 63.50, H, 5.05, F, 5.29, N, 3.90, Found: C, 63.52, H, 5.04, F, 5.30, N, 3.91.

Methyl ester of N- (3', 4'-difluoro-*trans*-cinnamoy1)-3-(3, 4-dihydroxyphenyl)-L-alanine (10). Yield: 331.36 mg (87%). IR cm^{-1}: 1756.35 (CO ester); 1680.60 (CO amide). MS (ESI) m/z: 386.11 $[M+Na]^{+}$. ^{1}H RMN (MeOD, 500Mhz): 2.96 (dd, J=7.4, 13.8 Hz, 1H, CH_2), 3.09 (dd, J=6.73, 13.8 Hz, 1H, CH_2), 4.74 (t, J= 7.1 Hz, 1H, CH), 6.55 (d, J=15.70, CH); 6.63 -7.30 (m, $6H_{Ar}$), 7.45 (d, J=15.7Hz, CH). ^{13}C RMN (MeOD, 125Mhz): δ 38.10 CH_2, 53.02 CH, 55.90 CH_3, 116.37 (2x CH), 117.06 CH, 117.28 (d, J=18.47 Hz, CH), 119.07 (d, J=17.32 Hz, CH), 121.58 CH, 126.13 (dd, J=3.25, 6.51 Hz, CH), 129.34 C, 134.08 (dd, J=3.24, 6.70 Hz, C), 140.11 CH, 145.36 C, 146.36 C, 150.93 (dd, J=13.10, 89.06 Hz, CF), 152.57 (dd, J=13.10, 92.80 Hz, CF), 167.76 CO, 173.58 CO. Anal. Calcd for: ($C_{19}H_{17}F_2NO_5$): C, 60.48, H, 4.54, F, 10.07, N, 3.71, Found: C, 60.50, H, 4.56, F, 10.09, N, 3.73.

Methyl ester of N- (4'-fluoro-*trans*-cinnamoy1)-3-(4-fluorophenyl)-L-alanine (11). Yield: 331 mg (96%). IR cm^{-1}: 1735.30 (CO ester); 1687.50 (CO amide). MS (ESI) m/z:368.12 $[M+Na]^{+}$. ^{1}H RMN (MeOD, 500 Mhz): 3.02 (dd, J=7.4, 13.8 Hz, 1H, CH_2), 3.20 (dd, J=6.73, 13.8 Hz, 1H, CH_2), 3.7 (s, 3H, CH), 3.8 (s, 3H, CH), 4.80 (t, J= 7.1 Hz, 1H, CH), 6.60 (d, J=15.70, CH), 6.99-7.30 (m, $8H_{Ar}$), 7.50 (d, J=15.7Hz, CH). ^{13}C RMN (MeOD, 125Mhz): δ 37.71 CH_2, 52.74 CH, 55.44 CH_3, 116.04 (d, J= 20.96 Hz, 2x CH), 116.75 (d, J=22.20 Hz, CH, CH), 120.99 (d, J=2.00 Hz, CH), 130.95 (d, J=8.50 Hz, 2xCH), 131.93 (d, J=8.16 Hz, 2xCH), 132.58 (d, J= 3.04 Hz, $C_)$, 134.08 (d, J=3.80 Hz, $C_)$, 141.24 CH, 162.38 (d, J=215.12 Hz, CF), 164.32 (d, J=219.18 Hz, CF), 168.20 CO, 173.29 CO. Anal. Calcd for: ($C_{19}H1_7F_2NO_3$): C, 66.08, H, 4.96, F, 11.00, N, 4.06, Found: C, 62.07, H, 4.94, F, 11.05, N, 4.05.

Methyl ester of N- (4'-fluoro-*trans*-cinnamoy 1)-3-(3, 4-difluorophenyl)-L-alanine (12). Yield: 323.16 mg, (89%). IR cm^{-1}: 1745.35 (CO ester); 1687.60 (CO amide). MS (ESI) m/z:386.11 $[M+Na]^{+}$.^{1}H RMN (MeOD, 500 Mhz): 3.01 (dd, J=7.4, 13.8 Hz, 1H, CH_2), 3.20 (dd, J=6.73, 13.8 Hz, 1H, CH_2), 4.79 (t, J= 7.1 Hz, 1H, CH), 6.99 (d, J=15.70, CH); 7.00-7.33 (m, $7H_{Ar}$), 7.45 (d, J=15.7Hz, CH). ^{13}C RMN (MeOD, 125 Mhz): δ 37.68 CH_2, 52.78 CH, 55.45 CH_3, 116.06 (d, J= 22.67 Hz, CH, CH), 117.16 (d, J=18.47 Hz, CH), 118.84 (d, J=16.80 Hz, CH_{Ar}), 12245 (d, J=1.98 Hz, CH_{Ar}), 126.13 (dd, J=3.25, 6.51 Hz, CH_{Ar}), 131.99 (d, J=7.73

Hz, 2xCH_{Ar}), 133.80 (q, J= 4.47, Hz, $C_)$, 134.08 (d, J=3.24 Hz, $C_)$, 140.19 CH, 150.93 (dd, J=13.10, 87.06 Hz, CF), 152.57 (dd, J=13.10, 90.80 Hz, CF), 164.19 (d, J=243.91 Hz, CF), 167.74 CO, 173.23 CO. Anal. Calcd for: ($C_{19}H_{16}F_3NO_3$): C, 62.81, H, 4.44, F, 15.69, N, 3.86, Found: C, 62.79, H, 4.45, F, 15.05, N, 3.84.

Methyl ester of N- (3', 4'-dihydroxy-*trans*-cinnamoy1)-3-(4-fluorophenyl)-L-alanine (13). Yield: 326.80 mg (91%). IR cm^{-1}: 1760.38 (CO ester); 1686.60 (CO amide). MS (ESI) m/z:382.12 $[M+Na]^+$. ^1H RMN (MeOD, 500 Mhz): 3.01 (dd, J=7.4, 13.8 Hz, 1H, CH_2), 3.20 (dd, J=6.73, 13.8 Hz, 1H, CH_2), 4.80 (t, J=7.1 Hz, 1H, CH), 6.48 (d, J=15.70, CH), 6.7-7.23 (m, 7H_{Ar}), 7.35 (d, J=15.7Hz, CH). ^{13}C RMN (MeOD, 125 Mhz): δ 37.73 CH_2, 52.71 CH, 55.45 CH_3, 115.12 (2x CH), 116.03 (d, J=18.18 Hz, CH), 116.45 CH, 117.56 CH, 122.24 CH, 122.95 (J= 73.94, CH), 128.18 C, 131.94 (d, J=7.19 Hz, $CH_)$, 134.19 (d, J=2.9 Hz, C), 143.12 CH, 146.73 C, 148.45 C, 164.16 (d, J=242.94, CF), 169.02 CO, 173.45 CO. Anal. Calcd for: ($C_{19}H_{18}FNO_5$): C, 63.50, H, 5.05, F, 5.29, N, 3.90, Found: C, 63.49, H, 5.06, F, 5.30, N, 3.89.

Table 2. *Yields obtained with molecules tested.*

Molecule	5	6	7	8	9	10	11	12	13
Yield (%)	91	77	80	90	80	87	96	89	91

3.2. Antioxydant Activity

The following tables show the IC_{50} and EC_{50} of molecules tested and reference molecules used in triplicate (n=3).
AcA: ascorbic acid; Quer: quercetine

Table 3. *IC_{50} of products tested.*

Molecule	5	6	7	8	9	10	11	12	13	AAc	Quer
IC_{50} (ug/ml)	3.46±0.03	3.04±0.01	4.23±0.02	5.10±0.06	1.55±0.17	6.023±0.07	2.49±0.06	14.49±0.10	9.86±0.16	2.29±0.03	1.20±0.01

Table 4. *EC_{50} of substances tested.*

Molecule	5	6	7	8	9	10	11	12	13	AAc	Quer
EC_{50} (g/mol)	0.34	0.29	0.41	0.50	0.15	0.59	0.24	0.70	0.97	0.22	0.11

4. Discussion

The coupling reaction using HOBT.H_2O, EDCI.HCl / DIPEA permited to synthesis *trans*-(-)-clovamide derivatives. The best yield was obtained with molecule 11 (96%) followed by molecules 5 and 13 (91% respectively) while molecule 6 gave a modest yield (77%).

The nine synthetic products were tested at concentrations between 1 and 10µg/ml and all of them showed antioxydant activity.

Molecule 9 is the product with the best antioxydant activity compared to the other products tested, with the lowest IC_{50} of 1.55 ± 0.17 µg / ml. This molecule is even more active than ascorbic acid with an IC_{50} of 2.29 ± 0.03 µg / ml. In addition, its antioxidant capacity is close to that of quercetin for which the IC50 of the absorbance of the radical is 1.20 ± 0.005 µg / ml. Indeed, the calculation of the IC_{50} confirmed that the antioxidant activity of molecule 9 (EC_{50}= 0.15) was close to that of Quercetin (EC_{50}= 0.11).

Molecules 8 and 13 have the same general formula but do not have the same antioxydant activity, 13 being less active than 8, which impels us to say that the fluorine position change on R1 and R4 plays on the antioxidant activity; the activity is higher when the fluorine is positioned on R4.

The molecule 9 being substituted with OH groups on R1, R2, R4 and R5 and H on R3 is more active, followed by 11 which carries OH on R4 and R5, fluorine on R2 and H on R1 and R3; 6 carries the OH in R2, R1 and R3 and the group OCH3 on R4 and R5; 5 carries OH on R1, R2 and R4 the OCH3 on R5 and H on R3; 7 carries OH on R1 and R2, OCH3 on R4 and R5 and H on R3; 8 carries OH on R1 and R2, H on R3 and R5 and F on R4; 10 carries OH on R1 and R2, H on R3 and F on R4 and R5; 12 carries Fluorine on R2, H on R1 and R3, OH on R4 and OCH3 on R5 and molecule 13 which carries F on R2, H on R1, OH on R3 and OCH3 on R4 and R5 is less active.

At the concentrations at which the references were tested, the products studied generally exhibited significant antioxidant power on the DPPH radical ($P<0.05$).

The molecules tested showed a greater activity compared to the plant extracts previously studied [11, 12].

From the results obtained in the present study, it is evident that the interaction of a potential antioxidant with DPPH• depends on its structural conformation.

Also, it is known that polyphenols have a higher antioxidant (antiradical) activity than monophenols [13]. For example, caffeic acid is a more efficient antiradical compound than coumaric acid, its monophenol counterpart. Gallic acid, a triphenol, is more efficient than protocatechic acid, its diphenol counterpart [13]. The compounds whose second hydroxyl group is in the ortho or para position have a higher activity than when it is meta. The efficiency of ortho and para diphenols is in part due to the stabilisation of the aryloxyl radical by hydrogen bonding or by regeneration of another diphenol [13].

In this work, the antioxidant effect may also be associated with the presence of the phenolic unit. Other functional groupings could also play a role in this activity.

In opposite to this advanced work, many authors reported antioxidant effects of African plants extracts without testing the pure molecules [13-15].

5. Conclusions

In this study, was prepared a small set of new nitrogen heterocycles derivatives of *trans*

(-) clovamide using a flexible chemistry. Nine new derivatives were prepared with good yield. The antioxidant activity of these different molecules was evaluated by the DPPH test. This study showed that compound 9 with an IC_{50} of 1.55 µg/ml has the activity close to that of quercetin (IC_{50} = 1.20µg/ml).

While antioxydant activity of other new molecules are being tested by our team, it would also be interesting to study the various products tested further by conducting a toxicological study and using cancer models *in vitro* and *in vivo*.

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