ORIGINAL ARTICLE

NEW ARGININE DERIVATIVES - SYNTHESIS AND BIOLOGICAL EVALUATION

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Abstract

A novel series of L-arginine derivates with imine structure (Schiff bases) were synthetized and the structure was confirmed using spectral methods: infrared (IR), nuclear magnetic resonance (^{1}H-NMR) and high resolution mass spectroscopy (HR-MS). The antioxidant potential was evaluated *in vitro* using the DPPH and ABTS radical scavenging assays. Most of the synthesized compounds revealed a better antioxidant activity in comparison with L-arginine. The most active compounds were the derivatives that have -NO_2 (**2e**, **2g**) and di-OH (**2k**, **2l**) as substituents on the aromatic ring.

Rezumat

A fost sintetizată o nouă serie de derivați ai L-argininei cu structură iminică (baze Schiff), iar structura compușilor a fost confirmată prin metode spectrale: spectroscopie în infraroșu (IR), spectroscopie de rezonanță magnetică nucleară (^{1}H-RMN) și spectroscopie de masă de înaltă rezoluție (HR-MS). Potențialul antioxidant s-a evaluat *in vitro* pe baza testelor antiradicalice DPPH și ABTS. Cei mai mulți dintre compușii sintetizați au demonstrat o acțiune antioxidantă mai intensă decât a L-argininei. Cei mai activi au fost compușii care prezintă pe nucleul aromatic substituenții -NO_2 (**2e**, **2g**) și di-OH (**2k**, **2l**).

Keywords: L-arginine, imine structure, antiradical activity

Introduction

The chemical substances that have in their structure the imine bound (–CH=N-) are known as Schiff bases and they have shown a wide range of biological effects such as antioxidant, antipyretic, antiproliferative, anti-inflammatory, antifungal, antibacterial, antimalarial, and antiviral effects [1, 2]. L-arginine is classified as a semi-essential or conditionally essential amino acid, depending on the stage of development and on the individual state of health. For example, the infants cannot synthesize L-arginine in which case it becomes an essential amino-acid. There are also specific situations which require an increase in the synthesis of L-arginine such as burns, after certain surgeries and sepsis [3]. L-Arginine is a precursor of nitric oxide, being the only nutrient for endothelial cells in blood vessels needed for the production of endogenous nitric oxide (NO) [4]. It was also proved that exogenous L-arginine possesses superoxide scavenging activity and it is able to delay the cell-mediated breakdown of NO as well as to reduce the oxidation of lipoproteins [5].

In order to improve the antioxidant effects of L-arginine it has been synthetized and biologically evaluated a series of 13 imine derivatives of L-arginine.

Materials and Methods

The organic solvents (*p.a.* quality), aromatic aldehydes (4-chloro/4-fluoro/4-bromo/2-nitro/3-nitro/4-nitro/2-hydroxy/3-hydroxy/2-methoxy/2,3-dihydroxy/2,4-dihydroxy/2,4,6-trihydroxy-benzaldehyde) and the standard reagents used for the antioxidant assays were purchased from Sigma Aldrich Company and Fluka Company. All the solvents and the reagents were used without prior purification. For monitoring the synthesis there has been used thin layer chromatography (TLC) plates silica gel 60 F_{254} from Merck Company.

Synthesis of the imine derivatives of L-arginine. The synthesis of imine derivatives consists in the condensation of L-arginine (**1**) with different aromatic aldehydes which led to the corresponding imines (**2a-m**) (Figure 1).

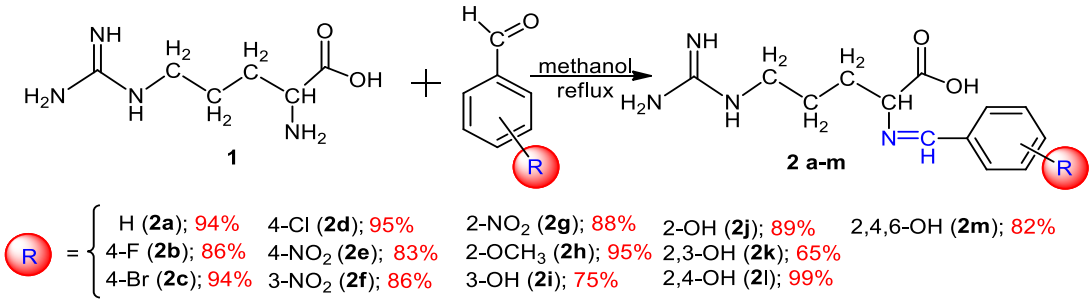

Figure 1.
The synthesis of the imine derivatives of arginine

General procedure for the synthesis of the imine derivatives of L-arginine. L-arginine (1 mmol, MW = 174.20 g/mol) was suspended in methanol and refluxed in order to increase the solubility of arginine and then the aromatic aldehyde (1 mmol) was slowly added under continuous stirring. The mixture was kept at reflux for 4-8 h until the completion of the reaction (TLC monitoring, using dichloromethane: methanol - 8:2, v/v, UV light at 254 nm). At the end of the reaction the mixture was a clear solution, colourless or coloured in different shades. The solvent was removed under reduced pressure and the residue was precipitated using petroleum ether or chloroform [6-8].

Characterization by spectral methods. The FT-IR spectra of the compounds (**2a-m**) were recorded using an ABB-MB 3000 FT-IR MIRacle^{TM} Single Bounce ATR-crystal ZnSe system, over a 500-4000 cm^{-1} range, after 16 scans at a resolution of 4 cm^{-1}. The spectra processing was carried out with the Horizon MB^{TM} FTIR Software. The ^{1}H-NMR spectra were registered using a Bruker Avance 400 MHz Spectrometer (Germany) using tetramethylsilane as internal standard and deuterated methanol (CD_3OD) or deuterated dimethyl sulfoxide (DMSO-d6) as solvents, depending on the solubility of the synthetized products. The chemical shifts were recorded in δ values (ppm). The mass spectra were registered using BrukerMaXis Ultra-High Resolution Quadrupole Time-of-Flight Mass Spectrometer.

Biological Evaluation

The evaluation of the antioxidant potential was achieved by determining the antiradical effect using the free radical DPPH (2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl) and radical cation ABTS$^{\bullet+}$ (2,2'-azino-bis(3-ethyl-benzthiazoline-6-sulphonic acid) assays. The results were expressed as EC_{50} value which represents the concentration where half of the substrate is being reduced by the substances with antioxidant capacity.

The DPPH Radical Scavenging Assay

The L-arginine-derivatives and L-arginine were dissolved in DMSO and water respectively to obtain a stock solution of 10 mg/mL, according to the procedure described in the literature [9-11] with slight modifications. From the stock solution different volumes (50 µL, 100 µL, 150 µL, 200 µL) were taken and diluted with methanol for obtaining 200 µL solution. After that, 2800 µL of methanol solution of DPPH (0.1 mM, A_{517nm} = 1.0 ± 0.05) were added and the mixture was left at room temperature, in the dark, for 30 min. The absorbance was measured at 517 nm against a blank solution (methanol). The DPPH scavenging effect (I%) was calculated using the following formula: I% = (A_0 – A_s/A_0) x 100, where A_0 is the absorbance of 0.1 mM DPPH solution and A_s is the absorbance of the sample after 30 min. The effective concentration 50 (EC_{50}) for each compound was determined using linear regression analysis and ascorbic acid (10 mg/mL) was used as positive control. All experiments were performed in triplicate and the results were statistically analysed using the analysis of variance (ANOVA) ($p < 0.050$) and were expressed as arithmetic average ± standard deviation (SD) [12].

The ABTS Radical Scavenging Assay

The ABTS cation radical (ABTS$^{\bullet+}$) was produced by reacting of 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)diammonium salt (ABTS, 7 mM) with potassium persulfate (2.45 mM), keeping the mixture in dark at room temperature for 16 h. Before starting the experiment, the ABTS$^{\bullet+}$ solution was diluted with ethanol to obtain a solution with absorbance value of 0.700 ± 0.020 at 734 nm [13, 14]. Similar to the DPPH radical scavenging assay, a stock solution of L-arginine derivatives and L-arginine with the concentration of 10 mg/mL in DMSO and water respectively was made. From the stock solution different volumes (5 µL, 10 µL, 20 µL, 25 µL) were measured, diluted with DMSO to 25 µL and after that 1975 µL of ABTS$^{\bullet+}$ solution were added. The absorbance was measured after 6 min at 734 nm and the radical scavenging ability was determined according to the following equation: I% = (A_0 – A_s/A_0) x 100, where A_0 is the absorbance before adding the sample and A_s is the absorbance registered after 6 min. The effective concentration 50 (EC_{50}) for each compound was

determined using linear regression analysis and ascorbic acid (10 mg/mL) was used as positive control. All experiments were performed in triplicate and the results were statistically analysed using the analysis of variance (ANOVA) ($p < 0.050$) and were expressed as arithmetic average ± standard deviation (SD) [12, 15].

Results and Discussion

Physico-chemical and spectral characterization

The L-arginine derivatives (**2a-m**) are crystalline powders, which have a coloration that varies from white to bright brown. The compounds are soluble in DMFA (dimethylformamide), DMSO (dimethylsulfoxide) and distilled water; sparingly soluble in acetone, absolute ethanol, propanol and methanol; insoluble in chloroform, benzene, dioxane, diethyl ether. The melting point (m.p.), (°C), yield (η, %) and molecular weight expressed as $[M+H]^+$ values (calculated and found) are presented in Table I.

In the FT-IR spectra the appearance of the characteristic band of the -N=CH- at 1558-1588 cm^{-1} confirms the successfully formation of the imine derivatives of L-arginine. The characteristic bands of the aromatic ring appeared in the range of 2982-2964 cm^{-1} and between 1107-1015 cm^{-1}, and the phenyl ring substituents were observed at 3482-3483 cm^{-1} (OH), 3132 cm^{-1} (OCH_3), 1369-1345 cm^{-1} (NO_2), 1154 cm^{-1} (C-F), 762 cm^{-1} (C-Cl), 632 cm^{-1} (C-Br). The formation of imine bound (-CH=N-) has been proved by the ^{1}H-NMR spectral data, the characteristic proton resonates as a singlet, between 8.56-8.08 ppm (Table II). The ^{1}H-NMR spectral data coupled with mass spectra strongly support the proposed structures of the all synthesized compounds.

Table I

Physico-chemical characteristics of the L-arginine derivatives (**2a-m**)

No.	R	η (%)	m.p. (^{0}C)	$[M+H]^+$ calculated	$[M+H]^+$ found
2a	H	93.87	140	262.32116	262.32109
2b	4-F	85.63	200	280.30234	280.30232
2c	4-Br	93.95	202	341.22043	341.22039
2d	4-Cl	94.89	220-224	296.75032	296.75030
2e	4-NO_2	82.57	90-95	307.32031	307.32029
2f	3-NO_2	86.31	207-214	307.32347	307.32343
2g	2-NO_2	88.07	110	307.32458	307.32455
2h	2-OCH_3	94.90	192-198	292.35187	292.35179
2i	3-OH	74.97	110	278.32007	278.32003
2j	2-OH	88.75	200-203	278.32115	278.32114
2k	2,3-OH	64.98	95-98	294.32112	294.32107
2l	2,4-OH	98.76	290-295	294.32019	294.32014
2m	2,4,6-OH	82.18	140	310.32001	310.32007

Table II

Spectral characteristics of the L-arginine derivatives (**2a-m**)

No	^{1}H-NMR signals δ (ppm)	FT-IR characteristic band (cm^{-1})
2a	8.41 (s, 1H, CH=N), 7.95-7.86 (m, 2H, Ar-H), 7.56 - 7.50 (m, 3H, Ar-H), 3.97 (dd, *J* = 7.5, 5.7 Hz, 1H, CH-COOH), 3.31 (t, *J* = 7.0 Hz, 2H, CH_2-NH), 2.20 - 1.92 (m, 2H, CH_2-CH), 1.73 (p, *J* = 7.4 Hz, 2H, CH_2-CH_2)	3375 (-NH_2), 3139 (-NH-), 2962 (CH_{Ar}), 1643 (-CO), 1558 (-C=NH-), 1326 (C-N), 1126 (-C-C-C-), 1041 (CH_{Ar})
2b	8.34 (s, 1H, CH=N), 7.87-7.72 (m, 2H, Ar-H), 7.13 (t, J = 8.7 Hz, 2H, Ar-H), 4.08 (dd, J = 8.2, 5.3 Hz, 1H, CH-COOH), 3.29-3.22 (m, 2H, CH_2-NH), 2.06-1.81 (m, 2H, CH_2-CH), 1.65-1.50 (m, 2H, CH_2-CH_2)	3332 (-NH_2), 3147 (-NH-), 2968 (CH_{Ar}), 1643 (-CO), 1558 (-C=NH-), 1154 (C-F), 1126 (-C-C-C-), 1042 (CH_{Ar})
2c	8.27 (s, 1H, CH=N), 7.72 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.58 (d, *J* = 8.4 Hz, 2H, Ar-H), 3.87 (t, *J* = 7.5, 1H, CH-COOH), 3.23-3.10 (m, 2H, CH_2-NH), 2.04-1.80 (m, 2H, CH_2-CH), 1.69-1.63 (m, 2H, CH_2-CH_2)	3325 (-NH_2), 3139 (-NH-), 2964 (CH_{Ar}), 1689 (-CO), 1577 (-C=NH-), 1126 (-C-C-C-), 1048 (CH_{Ar}), 632 (C-Br)
2d	8.30 (s, 1H, CH=N), 7.81 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.44 (d, *J* = 8.4 Hz, 2H, Ar-H), 3.89 (t, *J* = 7.5, 1H, CH-COOH), 3.23 (t, *J* = 7.1 Hz, 2H, CH_2-NH), 2.05-1.85 (m, 2H, CH_2-CH), 1.73-1.57 (m, 2H, CH_2-CH_2)	3325 (-NH_2), 3139 (-NH-), 2974 (CH_{Ar}), 1689 (-CO), 1588 (-C=NH-), 1126 (-C-C-C), 1054 (CH_{Ar}), 762 (C-Cl)
2e	8.54 (s, 1H, CH=N), 8.37-8.31 (m, 2H, Ar-H), 8.09 -8.03 (m, 2H, Ar-H), 4.24 (dd, *J* = 8.0, 5.2 Hz, 1H, CH-COOH), 3.35-3.21 (m, 2H, CH_2-NH), 2.14-2.02 (m, 1H, CH_2-CH), 2.02-1.92 (m, 1H, CH_2-CH), 1.71-1.58 (m, 2H, CH_2-CH_2)	3440 (-NH_2), 3330 (-NH-), 2958 (CH_{Ar}), 1635 (-CO), 1568 (-C=NH-), 1369 (NO_2), 1124 (-C-C-C), 1107 (CH_{Ar})
2f	8.68 (t, J = 1.9 Hz, 1H, Ar-H), 8.53 (s, 1H, CH=N), 8.35 (ddd, J = 8.2, 2.4, 1.1 Hz, 1H, Ar-H), 8.19 (dt, J = 7.8, 1.3 Hz, 1H, Ar-H), 7.72 (t, J = 8.0 Hz, 1H, Ar-H), 4.23 (dd, J = 8.1, 5.2 Hz, 1H, CH-COOH), 3.35 – 3.31 (m, 2H, CH_2-NH), 2.27 – 1.88 (m, 2H, CH_2-CH), 1.86 – 1.43 (m, 2H, CH_2-CH_2)	3271 (-NH_2), 3139 (-NH-), 2981 (CH_{Ar}),1635 (-CO), 1566 (-C=NH-), 1345 (NO_2), 1210 (-C-C-C), 1063 (CH_{Ar})
2g	8.50 (s, 1H, CH=N), 8.00 (q, J = 7.7 Hz, 1H, Ar-H), 7.78 (t, J = 7.4 Hz, 1H, Ar-H), 7.53 (ddt, J = 37.2, 22.0, 7.8 Hz, 1H, Ar-H), 7.26 (h, J = 7.5 Hz, 1H, Ar-H), 3.77 (t, J = 6.4 Hz, 1H, CH-COOH), 3.27- 2.93 (m, 2H, CH_2-NH), 1.90 (dt, J = 13.3, 6.5 Hz, 1H, CH_2-CH), 1.71 (dt, J = 16.4, 8.1 Hz, 1H, CH_2-CH), 1.49 (p, J = 7.9, 7.5 Hz, 2H, CH_2-CH_2)	3325 (-NH_2), 3147 (-NH-), 2980 (CH_{Ar}), 1639 (-CO), 1566 (-C=NH-), 1349 (NO_2), 1215 (-C-C-C), 1015 (-CH_{Ar})
2h	8.56 (s, 1H, CH=N), 7.86-7.79 (m, 1H, Ar-H), 7.45-7.36 (m, 1H, Ar-H), 7.13- 6.93 (m, 2H, Ar-H), 3.84 (s, 3H, OCH_3), 3.66 (t, J = 6.9 Hz, 1H, CH-COOH), 3.17-3.02 (m, 2H, CH_2-NH), 1.98-1.82 (m, 2H, CH_2-CH), 1.74-1.57 (m, 2H, CH_2-CH_2)	3271 (-NH_2), 3162 (-NH-), 3132 (-OCH_3), 2968 (CH_{Ar}), 1681 (-CO), 1566 (-C=NH-), 1110 (-C-C-C-), 1049 (CH_{Ar})

No	^{1}H-NMR signals δ (ppm)	FT-IR characteristic band (cm^{-1})
2i	8.24 (s, 1H, C<u>H</u>=N), 7.21-7.13 (m, 3H, Ar-H), 6.88 (ddd, J = 7.6, 2.5, 1.8 Hz, 1H, Ar-H), 4.04 (dd, J = 8.2, 5.2 Hz, 1H, C<u>H</u>-COOH), 3.29-3.22 (m, 2H, C<u>H$_2$</u>-NH), 2.11-1.83 (m, 2H, C<u>H$_2$</u>-CH), 1.63-1.49 (m, 2H, C<u>H$_2$</u>-CH$_2$)	3482 (OH), 3334 (-NH_2), 3157 (-NH-), 2974 (CH_{Ar}), 1662 (-CO), 1558 (-N=CH-), 1124 (-C-C-C-), 1084 (CH_{Ar})
2j	8.43 (s, 1H, C<u>H</u>=N), 7.37 (dd, *J* = 7.7, 1.8 Hz, 1H, Ar-<u>H</u>), 7.28 (ddd, *J* = 8.6, 7.2, 1.7 Hz, 1H, Ar-<u>H</u>), 6.83-6.74 (m, 2H, Ar-<u>H</u>), 3.79 (t, *J* = 6.4 Hz, 1H, C<u>H</u>-COOH), 3.10 (q, *J* = 6.5 Hz, 2H, C<u>H$_2$</u>-NH), 2.05-1.86 (m, 1H, C<u>H$_2$</u>-CH), 1.83-1.67 (m, 1H, CH$_2$-CH), 1.51 (q, *J* = 7.4 Hz, 2H, C<u>H$_2$</u>-CH$_2$)	3482 (OH), 3409 (-NH_2), 3311 (-NH-), 2971 (CH_{Ar}), 1647 (-CO), 1585 (-N=CH-), 1132 (-C-C-C-), 1078 (CH_{Ar})
2k	8.33 (s, 1H, C<u>H</u>=N), 6.86-6.75 (m, 2H, Ar-H), 6.53 (t, *J* = 7.8 Hz, 1H, Ar-H), 4.02 (t, *J* = 6.3 Hz, 1H, C<u>H</u>-COOH), 3.22 (t, *J* = 7.1 Hz, 2H, C<u>H$_2$</u>-NH), 1.97 (dt, *J* = 15.0, 7.6 Hz, 2H, C<u>H$_2$</u>-CH), 1.68 (p, *J* = 7.5 Hz, 2H, C<u>H$_2$</u>-CH$_2$)	3483 (OH), 3408 (-NH_2), 3324 (-NH-), 2951 (CH_{Ar}), 1651 (-CO), 1563 (-N=CH-), 1172 (-C-C-C), 1080 (CH_{Ar})
2l	8.08 (s, 1H, C<u>H</u>=N), 7.00 (d, J = 8.7 Hz, 1H, Ar-<u>H</u>), 6.01 (dd, J = 8.6, 2.2 Hz, 1H, Ar-<u>H</u>), 5.93 (d, J = 2.2 Hz, 1H, Ar-<u>H</u>), 3.92-3.64 (m, 1H, C<u>H</u>-COOH), 3.06 (q, J = 10.6, 7.0 Hz, 2H, C<u>H$_2$</u>-NH), 1.96-1.64 (m, 2H,C<u>H$_2$</u>-CH), 1.58-1.24 (m, 2H, C<u>H$_2$</u>-CH$_2$)	3483 (OH), 3421 (-NH_2), 3346 (-NH), 2974 (CH_{Ar}), 1651 (CO), 1563 (-N=CH), 1172 (-C-C-C), 1065 (CH_{Ar})
2m	8.08 (s, 1H, C<u>H</u>=N), 6.17 (s, 2H, Ar-<u>H</u>), 3.87-3.52 (m, 1H, C<u>H</u>-COOH), 3.14-3.02 (m, 2H, C<u>H$_2$</u>-NH), 1.86-1.57 (m, 2H,C<u>H$_2$</u>-CH), 1.34-1.14 (m, 2H, C<u>H$_2$</u>-CH$_2$)	3483 (OH), 3375 (-NH_2), 3150 (-NH-), 2982 (CH_{Ar}), 1680 (-CO), 1558 (-C=NH-), 1126 (-C-C-C-), 1043 (CH_{Ar})

The antioxidant activity

The DPPH Radical Scavenging Assay. The purple free radical DPPH (2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl) is a stable compound that can be scavenged by antioxidants by reduction to 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazine), a yellow product visible at 517 nm. The compound which has the smallest value of EC_{50} has an important scavenging activity. From the results obtained (Table III) it was observed that the most active compounds, for all tested concentrations (0.16 mg/mL, 0.32 mg/mL, 0.49 mg/mL and 0.64 mg/mL), were the compounds obtained by condensation of L-arginine with 4-nitrobenzaldehyde (**2e**), 2-nitro-benzaldehyde (**2g**), 2,3 dihydroxybenzaldehyde (**2k**) and 2,4-dihydroxy benzaldehyde (**2l**).

Table III

The DPPH radical scavenging ability (EC_{50}, mg/mL) of the tested compounds (**2a-m**)

No	EC_{50} mg/mL	No	EC_{50} mg/mL	No	EC_{50} mg/mL
L-Arg	1.47 ± 0.24	2e	0.02 ± 0.23	2j	0.97 ± 0.72
2a	0.33 ± 0.16	2f	0.14 ± 0.25	2k	0.03 ± 0.38
2b	0.80 ± 0.96	2g	0.03 ± 0.41	2l	0.03 ± 0.51
2c	0.56 ± 0.23	2h	1.15 ± 0.84	2m	0.85 ± 0.06
2d	1.09 ± 0.09	2i	1.23 ± 0.22	AA	0.006 ± 0,023

L-Arg: L-arginine; AA: ascorbic acid; Data are mean ± SD (n = 3, $p < 0.05$)

For example, at the concentration of 0.32 mg/mL these derivatives were 68 times (**2e),** 56 times **(2g),** 50 times (**2k)** and 59 times (**2l)** more active than L-arginine (L-Arg). Compared to ascorbic acid (AA), used as a positive control, all tested compounds were less active in similar conditions.

The ABTS Radical Scavenging Assay. This method measures the ability of compounds to scavenge the $ABTS^{\cdot+}$. From the results obtained (Table IV) it was observed that the most active compounds were **2e**, **2g**, **2k**, **2l**, similar to results of the DPPH assay. These derivatives are being approximately 60 times more active than L-arginine (L-Arg), but in the same time they are less active than ascorbic acid (AA), used as positive control, in similar experimental concentrations.

Table IV

The ABTS radical scavenging ability (EC_{50}, mg/mL) of the tested compounds (**2a-m**)

No	EC_{50} mg/mL	No	EC_{50} mg/mL	No	EC_{50} mg/mL
L-Arg	1.78 ± 0.27	2e	0.67 ± 0.17	2j	1.32 ± 0.22
2a	1.24 ± 0.60	2f	1.23 ± 0.20	2k	0.74 ± 0.38
2b	1.59 ± 0.07	2g	0.45 ± 0.14	2l	0.59 ± 0.01
2c	0.95 ± 0.35	2h	1.57 ± 0.20	2m	1.39 ± 0.26
2d	1.72 ± 0.48	2i	1.24 ± 0.09	AA	0.005 ± 0,014

L-Arg: L-arginine; AA: ascorbic acid; Data are mean ± SD (n = 3, $p < 0.05$)

It is also noted that the antiradical activity of the studied derivatives increases with the concentration, the highest inhibition effect being recorded at the concentration of 50 mg/mL in which the inhibition rate ranged from 27.11% (**2a**, R = H) up to 98.73% for (**2g**, R = 2-NO_2) and 99.63% (**2l**, R =2,4-OH).

Conclusions

There have been synthesized a number of 13 derivatives of L-arginine and the optimal conditions of reaction were established. The synthesized compounds have been characterized by their physical constants (melting point, yield and solubility in different organic solvents) and the chemical structure was confirmed using FT-IR, ^{1}H-NMR and HR-MS spectral data. The antioxidant effects of the synthesized derivatives were evaluated using two *in vitro* methods: the DPPH and the ABTS radical scavenging assays. All studied compounds showed a higher antioxidant capacity in comparison with L-arginine that means that all the chemical modifications made on the arginine scaffold were favourable in terms of biological action. Related to the influence of the radical which substitutes the aromatic ring it was observed that substitution with nitro in ortho and para position (**2g**, **2e**) and di-hydroxy (2,3-OH, **2k**; 2,4-OH, **2l**) has the most favourable influence.

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