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Synthesis, characterization and acute toxicity of new Schiff base derived from L-arginine and vanillin

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ABSTRACT

Condensation of L-arginine with 4-hydroxy-3-methoxy benzaldehyde (vanillin) yielded a novel Schiff base derivative of arginine in good yield. This new Schiff base was characterized by elemental analysis, IR and ^{1}H NMR spectroscopy. The toxicity of the compound was also assayed via the determination of their LD_{50} value by using Dixon method. Studied compound was found to have an LD_{50} of 718.6 mg/kg of body weight.

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1. Introduction

L-Arginine is a basic natural amino acid. It plays an important role in cell division, the healing of wounds, removing ammonia from the body, immune function, and the release of hormones [1-3]. It has high important in many diseases, low dose IV arginine has been shown to improve insulin sensitivity in obese, type 2 diabetic, and healthy subjects [4]. Arginine may also counteract lipid peroxidation and thereby reduce microangiopathic long-term complications of diabetes mellitus [5]. L-Arginine has been purported to have ergogenic potential [6]. L-Arginine stimulates the release of growth hormone [7], as well as the release of pancreatic insulin and glucagon and pituitary prolactin [8]. The antioxidant property of L-arginine has been well documented in several reports [5,8]. An interesting article by Grasemann *et al*. 2006 [9], has demonstrated an acute and transient improvement of pulmonary function in cystic fibrosis patients by a single inhalation of L-arginine [10].

Sarı and Gurkan synthesized a new Schiff bases derived from thiophene-2-carbaldehyde with some amino acids as DL-alanine, DL-valine and DL-phenylalanine, with a new alternative method [11]. Vanillin is used as a flavoring aromatic compound in foods and fragrance industries.

Synthetic vanillin is used as an intermediate in the chemical and pharmaceutical industries for the synthesis of herbicides and drugs [12]. Recent reports have shown that vanillin can act as an antioxidant improving the keeping quality of precooked dried cereal flakes [13] and afforded significant protection against protein oxidation and lipid peroxidation in rat liver mitochondria [14]. The aim of present work was synthesized novel arginin derivative byreaction of L-arginine with bioactive materials vanillin.

2. Experimental

2.1. Instrumentation

Infrared spectra (IR) were recorded as KBr discs in the range of 4000-400 cm^{-1} using Shimadzu FT-IR spectrophotometer in the Department of Chemistry, College of Education for Pure Sciences, University of Basrah, Iraq. ^{1}H NMR spectra were measured on a Bruker at 600 MHz, with TMS as internal reference at Konstanz University, Germany. Microanalysis for carbon, hydrogen and nitrogen were carried out by a Perkin-Elmer 240B Elemental Analyzer. Melting points were measured by a Philip Harris melting point apparatus and uncorrected.

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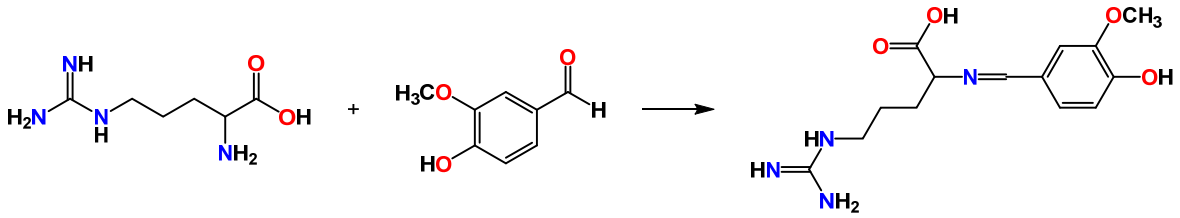
Table 1. Dixon values.

Second part of serial	+ K represented serial tests as follows				
	O	OO	OOO	OOOO	
XOOO	-0.157	-0.154	-0.154	-0.154	OXXX
XOOX	0.878	-0.861	-0.860	-0.860	OXXO
XOXO	0.701	0.737	0.741	0.741	OXOX
XOXX	0.084	0.169	0.181	0.186	OXOO
XXOO	0.305	0.372	0.380	0.381	OOXX
XXOX	-0.305	0.169	-0.144	-0.142	OOXO
XXXO	1.288	1.500	1.544	1.549	OOOX
XXXX	0.555	0.896	0.985	1.007	OOOO
	X	XX	XXX	XXXX	Second part of serial
	- K represented serial results as follows				



Scheme 1

2.2. Acute toxicity (LD_{50})

All experiments were performed on 10-14 weeks old male and female ratus-ratus/rats weighing 200-250 g at the time of treatment by using up-and-down method [15]. Male and female rats were injected intraperitonially with different doses of the arginin derivative after conducting series of test levels. With equal spacing between doses, a series of trails were carried out using this method: increased dose following a negative response and decreased dose following a positive response. Testing continued until chosen "nominal" sample size was reached. LD_{50} were determined after reading final result (response-dead (X) or non-response alive (O), then the following equation was applied

$$LD_{50} = XF + K \times d \quad (1)$$

where, LD_{50}: Median lethal dose; XF: Last dose administered; K = Value from Table 1; D = Difference between dose levels (Table 1) [15].

2.3. Synthesis

2.3.1. Synthesis of 5-carbamimidamido-2-[(4-hydroxy-3-methoxybenzylidene)amino]pentanoic acid

A solution of L-arginin (1.722 g, 10 mmole) in ethanol (15 mL) was added to a solution of 4-hydroxy-3-methoxy benzaldehyde (vanillin) (1.521 g, 10 mmole) in ethanol (15 mL). The mixture was refluxed for 4 hours with stirring. The resulting was a yellow solution allowed to cool and dried at room temperature, then re-crystallization to the precipitate with ethanol, brown solid was obtained by evaporation of ethanol during 24 hours, Scheme 1. Yield: 86%. M.p.: 116-118 °C. FT-IR (KBr, ν, cm^{-1}): 3107-3338 (OH, NH), 3060 (C-H, Aromatic), 2960, 2933 (C-H, Aliphatic), 1666 (C=N), 1639 (C=C), 1573, 1398 (COOH). ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 11.75 (s, 1H, COOH), 8.89 (s, 1H, Ar-OH), 8.43 (s, 1H, CH=N), 7.37-6.16 (m, 3H, Ar-H), 3.89-3.73 (m, 4H, NH + NH_2 + NH), 3.07 (s, 3H, OCH_3), 2.95 (t, 2H, CH_2-N), 2.44 (t, 1H, CH-COOH), 1.64-1.44 (m, 4H, $2CH_2$). Anal. calcd. for $C_{14}H_{20}N_4O_4$: C, 54.54; H, 6.54; N, 18.17. Found: C, 54.68; H, 7.02 ; N, 18.41%.

3. Results and discussion

3.1. Chemistry

Isolated yield, melting point, color and spectral data IR and ^{1}H NMR of synthesized compound was reported. The present work describes the synthesis of new Schiff base compound derived from L-arginine and vanillin to produce bioactive Schiff base, thus, the reaction of amino acid (L-arginine) with 4-hydroxy-3-methoxy benzaldehyde (vanillin) at 1:1 mole ratio afforded, after purification the new Schiff base derivative compound in good yield. IR spectra for synthesized compound displayed common features in certain regions and characteristic bands in the finger print and other regions. The IR spectra of new prepared compound show strong and broad bands in the range 3107-3338 cm^{-1} due to OH and (N-H) symmetrical and asymmetrical stretching vibration. The IR spectra of synthesized compound displays band at 1666 cm^{-1} is due to C=N group stretching vibration. The band at 1639 cm^{-1} was assigned to νC=C. The bands at 1573 and 1398 cm^{-1} were assigned to COOH.

The ^{1}H NMR spectra of studied synthesized compound was recorded in DMSO-d_6 solution and show all the expected protons with proper intensity ratio. In ^{1}H NMR spectrum of the synthesized compound, the single peaks attributed to methylene groups appeared at δ 1.64-1.44 ppm ($2CH_2$) and singlet peak at δ 3.07 ppm due to methoxy (OCH_3). The aromatic protons of compound appeared within the range δ 7.37-6.16 ppm. It is worthy to note that the protons of Ar-OH resonate as a singlet at δ 8.89 ppm. The proton of azomethine (CH=N) resonate as a singlet at δ 8.43 ppm, single peak attributed to hydroxyl group of carboxylic appeared at δ 11.75 ppm. Four groups of single peaks given by (C=NH), (C-NH_2) and (R_2-NH) on appeared at δ 3.89-3.73 ppm, ^{1}H NMR show triplet at δ 2.95 ppm attributed to CH_2-N group.

3.2. Median lethal dose (LD_{50})

Determination of the 50% of lethal dose (LD_{50}) of the studied compound *in-vivo* was detected in the rats by using the "up-and-down" procedure described by Dixon method [15]. In the experiment, we using 10 animals of white rats 10-14 weeks in age, Graded doses of injection to each one animal, a series of concentrations (500, 550, 600, 650, 700 and 750 mg/kg body weight) in 0.1 mL medical distilled water were administered and chosen with equal spacing (concentrations) between doses. Mortality was recorded after 24 hrs that each one animal treated with one dose and after 24 hrs was recorded as "O" if the animal lives and then increased the

treated dose. While "X" recorded for the death of animal and then decreased the dose according for the result of the animal the code which formed as being "OOXX" and according for Dixon value was get and the LD_{50} was determined according to the formula employed by Dixon method [Values: LD_{50} = XF+ K×d; LD_{50} = 700+0.372×50; LD_{50} = 718.6 mg/kg body weight; 1/10 LD_{50} = 71.86 mg/kg (1 kg = 6 rats) (Depending on the weight rat about 175 gram); 1/10 LD_{50}= 11.97 mg/rat (Depending on the weight rat 175 gram).

4. Conclusion

In conclusion, the present study reported the synthesis of new Schiff base derived from L-arginine and 4-hydroxy-3-methoxy benzaldehyde (vanillin) to yielded a novel derivative of arginine (5-carbamimid amido-2-[(4-hydroxy-3-methoxy benzylidene)amino]pentanoic acid) in good yield, which revealed moderate *in vivo* toxic effects by LD_{50} measurement.

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