https://doi.org/10.31925/farmacia.2019.5.6 **ORIGINAL ARTICLE**

ANTIOXIDANT AND ANTIMICROBIAL POTENTIAL OF NEW AZETIDIN-2-ONE OF FERULIC ACID

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Abstract

The objective of our study was to evaluate the antioxidant and antimicrobial potential for six new azetidin-2-one derivatives of ferulic acid. The *in vitro* antioxidant potential of the compounds was assessed by using the total antioxidant capacity and total reducing power assays. The antimicrobial activity was investigated using Gram positive bacteria (*Staphylococcus aureus* ATCC 25923), Gram negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) and pathogenic yeast (*Candida albicans* ATCC 90028, *Candida parapsilosis* ATCC 22019). Several of the synthesized compounds showed a good antioxidant activity, exceeding the ferulic acid antioxidant potential. All the investigated compounds proved active against Gram positive bacteria.

Rezumat

Obiectivul studiului a constat în evaluarea activităţii antioxidante şi antimicrobiene pentru derivaţii de azetidin-2-onă ai acidului ferulic. S-a evaluat potenţialul antioxidant *in vitro* al compuşilor prin determinarea capacităţii totale antioxidante şi determinarea puterii reducătoare. Activitatea antimicrobiană a fost studiată utilizând bacterii Gram pozitive (*Staphylococcus aureus* ATCC 25923), bacterii Gram negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) şi drojdii patogene (*Candida albicans* ATCC 90028, *Candida parapsilosis* ATCC 22019). Câţiva dintre compuşii sintetizaţi au prezentat o acţiune antioxidantă mai bună decât a acidului ferulic. În ceea ce priveşte acţiunea antimicrobiană, toate probele au fost active pe speciile Gram pozitive testate.

Keywords: azetidin-2-one, ferulic acid, antioxidant activity, antimicrobial activity

Introduction

An important risk factor in the pathogenesis of several chronic diseases is the oxidative stress involving free radicals [22]. Chemically synthesized antioxidants and secondary metabolites from plants are widely used in practice for their ability to trap free radicals [8, 10, 12, 16].

Ferulic acid and its derivatives have proven a powerful antioxidant activity in response to free radicals, acting synergistically with other antioxidants. Due to its structure, ferulic acid presents the ability to interrupt the propagation of free radical chain reactions, provide attack sites for free radicals and ensure protection against lipid peroxidation [8, 9, 17, 18].

On the other hand, the β-lactam heterocycles are substances which drew attention and interest of scientists for their potent antibacterial activity [1]. Presently, the research in this area is stimulated due to the development of bacterial resistance to the β-lactam antibiotics. Therefore, novel functionalized β-lactams are being explored [7, 9, 15]. The new interest has been focused on the synthesis and modification of β-lactam ring in order to obtain new derivatives with diverse biological activities.

Since cyclic amide (lactam) have similar structure with vitamin C, which is a cyclic ester (lactone), it would be challenging to study the antioxidant properties and also the antimicrobial properties of some derivatives which includes in their structure this lactam heterocycle.

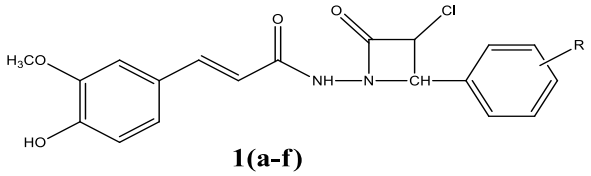
Hence, the aim of this study was to investigate the six new azetidin-2-one derivatives of ferulic acid for their antioxidant and antimicrobial activity.

Materials and Methods

Chemistry. The structure of the tested compounds, azetidin-2-one derivatives of ferulic acid, is presented in Table I.

Table I

Azetidin-2-one derivatives of ferulic acid (**1a-f**)



1(a-f)

Compound	-R	Compound	-R
1a	-H	**1d**	-NO_2(2)
1b	-F(4)	**1e**	-Br(4)
1c	-Cl(4)	**1f**	-OH(2)

Antioxidant tests

All the reagents used for both antioxidant assays were purchased from Sigma Aldrich Company and Fluka Company.

The total antioxidant capacity assay was assessed spectrophotometrically using phosphomolybdenum method [4, 16] with minor modifications. Stock solutions in DMSO (2 mg/mL) of the samples were obtained. Different volumes (10 µL, 20 µL, 40 µL, 60 µL) were measured, completed with DMSO up to 200 µL and shacked with 2000 µL of reagent solution (0.6M sulfuric acid, 28mM disodium phosphate and 4 mM ammonium heptamolybdate). The test tubes were incubated at 95°C for 90 min. After the cooling process at room temperature the samples were centrifuged at 4600 rpm for 15 min and the absorbance of the supernatant was measured at 695 nm using a blank (200 µL DMSO and 2000 µL reagent solution) [14]. Ascorbic acid (AA) was used as positive control. The effective concentration (EC_{50}) was calculated and expressed in µg/mL. All determinations were performed in triplicate and the values are expressed as mean ± SD (standard deviation).

The ferric reducing power assay is based on the coloured complex formation between antioxidant compound from the sample and potassium ferricyanide, trichloroacetic acid and ferric chloride [20, 21]. Stock solutions in DMSO (2 mg/mL) of the samples were obtained. The aliquots (1 mL) of different concentrations (0.25 mg/mL, 0.5 mg/mL, 1 mg/mL, 2 mg/mL) of the compounds, obtained from stock solutions, were added to 1 mL of sodium phosphate buffer (pH 6.6) and 1 mL of 1% potassium ferricyanide. The samples were incubated at 50°C for 20 min and then 1 mL of 10% tri-chloroacetic acid was added. After the centrifugation at 4500 rpm for 15 min the supernatant (1 mL) was mixed with 1 mL of double distilled water and 0.2 mL of 0.1% ferric chloride. The absorbance was read at 700 nm against the blank of the reference to standard (ascorbic acid) [21]. The results were expressed as EC_{50} values (µg/mL). All determinations were performed in triplicate and the values were expressed as mean ± SD.

Antimicrobial susceptibility tests

Microorganisms. It was studied the antimicrobial potential using Gram positive bacteria (*Staphylococcus aureus* ATCC 25923), Gram negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) and pathogenic yeasts (*Candida albicans* ATCC 90028, *Candida parapsilosis* ATCC 22019).

Disc-diffusion method. The disc-diffusion method (CLSI) was carried out by diluting in sterile 0.9% NaCl small amounts of each microbial culture until the turbidity was equivalent to McFarland standard no. 0.5 (106 CFU/mL). The obtained suspensions were incorporated in Mueller Hinton agar for bacteria (Oxoid) and in Mueller-Hinton agar for yeasts (Hi Media) in a 1:10 ratio, and then spread on sterile Petri plates (25 mL/Petri plate). Then, 0.1 mL of each compound were added into sterile stainless steel cylinders (5 mm internal diameter; 10 mm height), which were applied on the agar surface in Petri plates. As positive controls there were used commercially available discs of Ciprofloxacin (5 µg/disc), Fluconazol (25 µg/disc) and Nystatin (100 µg/disc). The plates were incubated at 37°C for 24 h (bacteria) and at 24°C for 48 h (yeasts), after that the diameters of inhibition zones were measured in mm, including the disc size [3, 5, 11].

Broth microdilution method. The compounds were tested for the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) against *Staphylococcus aureus* ATCC 25923. Serial double dilutions of each extract in Mueller Hinton broth (Oxoid) were inoculated with equal volumes of bacterial suspension (10^6 CFU/mL). The lowest concentration of extract for which the complete inhibition of visible growth was observed after 24 h incubation at 37°C, represents the MIC. In order to established the MBC values 0.01 mL of samples showing complete inhibition of visible growth on the surface of an agar plate were incubated 24 h at 35°C. The lowest concentration of extracts required to kill more than 99.9% of tested microorganisms represents the MBC [6, 11].

Results and Discussion

Total antioxidant capacity. Wishing to understand how different moieties have an effect on the anti-oxidant properties and the structure-activity relationship of different substituents, a series of new azetidin-2-one derivatives of ferulic acid (**1a-1f**) were investigated. The lactam ring may be responsible for enhancing the free radical scavenging activity. The results revealed that the newly synthesised compounds showed a greater antioxidant activity at low concentrations as compared to ferulic acid. The EC_{50} values of the tested compounds are presented in Table II. A low EC_{50} value indicates a higher total antioxidant capacity [16]. Good antioxidant activity showed the compounds resulting from the condensation reaction with 4-chlorobenzaldehyde **1c** (EC_{50} = 19.67 ± 0.07 µg/mL), 2-nitrobenzaldehyde **1d** (EC_{50} = 19.89 ± 0.05 µg/mL), 4-fluorobenzaldehyde **1b** (EC_{50} = 20.007 ± 0.03 µg/mL) and 2-hydroxybenzaldehyde **1f** (EC_{50} = 23.004 ± 0.06 µg/mL). These compounds are about 1.4 to 1.2 times more active than ferulic acid (EC_{50} = 27.62 ± 0.05 µg/mL) at the same concentration and so the results support the initial premise.

Table II

Total antioxidant capacity (EC_{50}, µg/mL) of the ferulic acid azetidin-2-one derivatives (**1a-f**)

Comp.	EC_{50} µg/mL	Comp.	EC_{50} µg/mL	Comp.	EC_{50} µg/mL
1a	31.55 ± 0.06	**1d**	19.89 ± 0.05	**FA**	27.62 ± 0.05
1b	20.01 ± 0.03	**1e**	39.30 ± 0.03	**AA**	5.16 ± 0.05
1c	19.67 ± 0.07	**1f**	23.00 ± 0.06		

FA - ferulic acid; AA - ascorbic acid; Data represent mean ± SD (n = 3, $p < 0.05$)

Ferric reducing power. The analysis of the results, presented in Table III, which are expressed as effective concentration 50 (EC_{50}), showed that the structural modulation of ferulic acid by obtaining the corresponding azetidine-2-one resulted mostly in the intensification of the ferric reducing power of ferulic acid. A small EC_{50} value indicated a higher reducing power [16]. Based on the structure activity relationships, it is indicated that the presence of substitution on the ferulic acid molecule and on the side chain phenyl ring influences the antioxidant potency of the molecule. The most active compounds were **1a** (EC_{50} = 25.08 ± 0.03 µg/mL), **1e** (EC_{50} = 26.71 ± 0.3 µg/mL), **1c** (EC_{50} = 27.61 ± 0.05 µg/mL) and **1b** (EC_{50} = 34.83 ± 0.02 µg/mL), the compounds being 1.52 to 1.1 times more active than ferulic acid (EC_{50} = 38.32 ± 0.05 µg/mL).

Table III

Ferric reducing power (EC_{50}, µg/mL) of the ferulic acid azetidin-2-one derivatives (**1a-f**)

Comp.	EC_{50} µg/mL	Comp.	EC_{50} µg/mL	Comp.	EC_{50} µg/mL
1a	25.08 ± 0.03	**1d**	43.86 ± 0.06	**FA**	38.32 ± 0.05
1b	34.83 ± 0.02	**1e**	26.71 ± 0.02	**AA**	5.16 ± 0.01
1c	27.61 ± 0.05	**1f**	66.94 ± 0.03		

FA - ferulic acid; AA - ascorbic acid; Data represent mean ± SD (n = 3, $p < 0.05$)

Table IV

Antibacterial and antifungal activities of the ferulic acid azetidin-2-one derivatives (**1a-f**)

Comp.	Diameter of inhibition zones (mm)				
	S. aureus **ATCC 25923**	***E. coli*** **ATCC 25922**	***P. aeruginosa*** **ATCC 27853**	***C. albicans*** **ATCC 90028**	***C. parapsilosis*** **ATCC 22019**
1a	16.0 ± 0.00	12.0 ± 0.00	16.0 ± 0.00	12.0 ± 0.00	15.0 ± 0.00
1b	15.0 ± 0.00	0	0	11.0 ± 0.00	12.3 ± 0.57
1c	13.3 ± 0.57	10.0 ± 0.00	0	15.0 ± 0.00	14.0 ± 0.00
1d	20.3 ± 0.57	10.0 ± 0.00	11.0 ± 0.00	11.0 ± 0.00	12.3 ± 0.57
1e	16.5 ± 0.50	15.0 ± 0.00	0	0	10.5 ± 0.50
1f	16.0 ± 0.00	14.0 ± 0.00	0	15.0 ± 0.00	10.0 ± 0.00
Ciprofloxacin (5 µg/disc)	26.7 ± 0.06	26.5 ± 0.50	30.0 ± 0.00	*NT	*NT
Fluconazol (25 µg/disc)	NT*	NT*	NT*	18.5 ± 0.50	18.5 ± 0.50
Nystatin (100 µg/disc)	NT*	NT*	NT*	18.5 ± 0.50	19.0 ± 0.00

*NT-not tested

Antimicrobial activity. In Table IV there are presented the diameters of the inhibition zones (in mm) for the tested compounds. The assays were carried out in triplicate and the results are expressed as means ± SD. A high activity was observed for all samples against Gram positive bacteria. For the compounds reported to have antimicrobial activity in the disc-diffusion method we have determined the MIC and MBC values against *S. aureus* ATCC 25923. The results are showed in Figure 1.

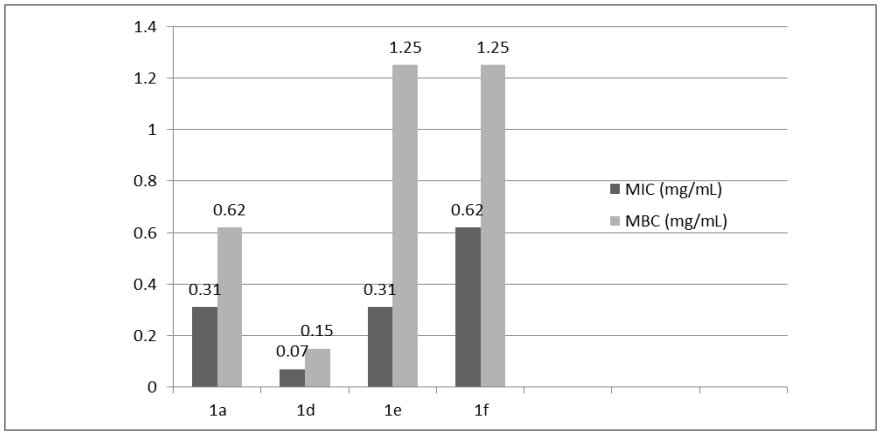


Figure 1.
MIC and MBC values of the ferulic acid azetidin-2-one derivatives against *S. aureus* ATCC 25923

Aiming to develop new systems with antibacterial or antifungal action we studied the antimicrobial activity against Gram positive and Gram negative bacteria and *Candida* spp. The antimicrobial assays showed an evident difference in terms of antibacterial and antifungal activity, implying that the tested compounds act differently on various types of micro-organisms. All new six tested compounds have good antibacterial activity against *S. aureus* ATCC 25923 and medium activity against *E. coli*. The most active compound against *S. aureus* proved to be **1d** (2-nitrobenzaldehyde) and against *E. coli* was **1e** (4-brombenzaldehyde). Against *P. aeruginosa* ATCC 27853 the tested compounds had no activity, except **1a** (H-benzaldehyde) and **1d** (2-nitrobenzaldehyde). Also, the tested compounds demonstrated a good activity against the *Candida* strains, except **1e** (4-brombenzaldehyde). The obtained results indicate that the new ferulic acid azetidin-2-one derivatives possess good antimicrobial properties. Nevertheless, ferulic acid acts against Gram-positive and Gram-negative bacteria [10] and lactam heterocycles have a potent antibacterial activity on Gram-positive bacteria [1]. The inherent variability and potency in the anti-microbial spectrum among the tested compounds may be explained by including the lactam hetero-cycle in the ferulic acid molecule and also by adding different substituents on the side chain phenyl ring [19]. In the quantitative assay all the tested compounds showed good MIC values for *S. aureus*. The MBC values for compounds **1a**, **1d**, **1f** were 2 times greater than the MIC values and for compound **1e** were 4 times greater.

Conclusions

Our results indicate that the new six azetidin-2-one derivatives of ferulic acid possess good antioxidant activity at low concentrations and good antimicrobial activity against Gram positive bacteria. While all the investigated compounds present biological activity, the intensity and efficiency depend on the nature of substituents attached to the phenyl ring. Regarding the antioxidant activity, the most active compounds proved to be **1c** (R = 4-Cl) and **1b** (R = 4-F), these compounds being more actives than ferulic acid in both antioxidant assays.

Considering the antimicrobial activity, the most active compound against *S. aureus* proved to be **1d** (R = 2-NO_2). The investigated derivatives present good *in vitro* antioxidant and antimicrobial potentials, but *in vivo* further studies still need to be performed in order to support the initial premise.

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