ORIGINAL ARTICLE

NEW THIAZOLIDINE-4-ONES OF FERULIC ACID WITH ANTIOXIDANT POTENTIAL

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Abstract

New thiazolidine-4-one derivatives of ferulic acid have been synthesized in one step ("one-pot") by cyclization of ferulic acid hydrazide with different aromatic aldehydes and mercaptoacetic acid. The structure of the synthesized compounds was proved by infrared (IR) and nuclear magnetic resonance (^{1}H-NMR) spectroscopy. The *in vitro* antioxidant potential of the synthesized compounds was evaluated according to the 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging assays. Some of synthesized compounds showed a good antioxidant activity which supports the favourable influence of the structural modulation on the antioxidant effects of the ferulic acid.

Rezumat

Au fost sintetizaţi noi derivaţi de tiazolidin-4-onă ai acidului ferulic, într-o singură etapă („*one pot*"), prin ciclizarea hidrazidei acidului ferulic cu diferite aldehide aromatice şi acid mercaptoacetic. Structura compuşilor sintetizaţi a fost dovedită prin spectroscopie în infraroşu (IR) şi de rezonanţă magnetică nucleară (^{1}H-RMN). S-a evaluat potenţialul antioxidant *in vitro* al compuşilor sintetizaţi pe baza testelor antiradicalice 1,1-difenil-2-picrilhidrazil (DPPH) şi acid 2,2'-azinobis-(3-etilbenzotiazoline-6-sulfonic (ABTS). Câţiva dintre compuşii sintetizaţi au prezentat o bună activitate antiradicalică, ceea ce susţine influenţa favorabilă a modulărilor structurale ale acidului ferulic, în ceea ce priveşte efectul antioxidant.

Keywords: ferulic acid, thiazolidin-4-one, antioxidant activity

Introduction

Thiazolidine-4-one derivatives represent one of the most studied classes of heterocyclic organic compounds due to their important biological effects. The first argument related to the importance of this heterocycle is penicillin and its derivatives that contain the thiazolidine ring in the beta-lactam structure. The compounds with thiazolidine structure have been reported for their antioxidant [1, 2, 3], antitumor [3, 4], antitubercular [5], antiinflammatory [6, 7] and analgesic [8] effects. The studies of structure-activity relationship have highlighted the influence of the substituents at C_2, N_3 and C_5 on biological properties of the thiazolidine-4-ones. It is known that electron-donating groups at N_3 improve the antiviral activity [9] and the electron withdrawing groups at N_3 prints simultaneously antibacterial and antiinflammatory properties [6].

On the other hand, ferulic acid has an important antioxidant effect and could be useful in the prevention and treatment of several diseases for which oxidative stress plays an important role like Alzheimer's disease, diabetes mellitus, cancer, hypertension and atherosclerosis [10].

In order to improve the antioxidant effects of ferulic acid, new thiazolidine-4-ones derivatives have been synthesized and biologically evaluated.

Materials and Methods

The aromatic aldehydes (2-hydroxy/2-nitro/4-nitro/2-methoxy/4-chloro/4-fluoro/4-bromo/2,3-dihydroxy/2,6-dichloro/4-dimethylamino/4-hydroxy-3-methoxi-benzaldehyde), mercaptoacetic acid, organic solvents (*p.a*. quality) 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. Thin layer

chromatography (TLC) plates silica gel 60 F_{254} from Merck Company were used for monitoring of the chemical reactions.

Synthesis of thiazolidine-4-ones of ferulic acid. The hydrazide of ferulic acid (3) has reacted with different aromatic aldehydes and mercaptoacetic acid in excess to give the corresponding thiazolidine-4-ones (4a-l) (Figure 1). Hydrazide (3) was obtained starting from ferrulic acid (1) which was converted to corresponding chloride (2) and then reacted with hydrazine hydrate.

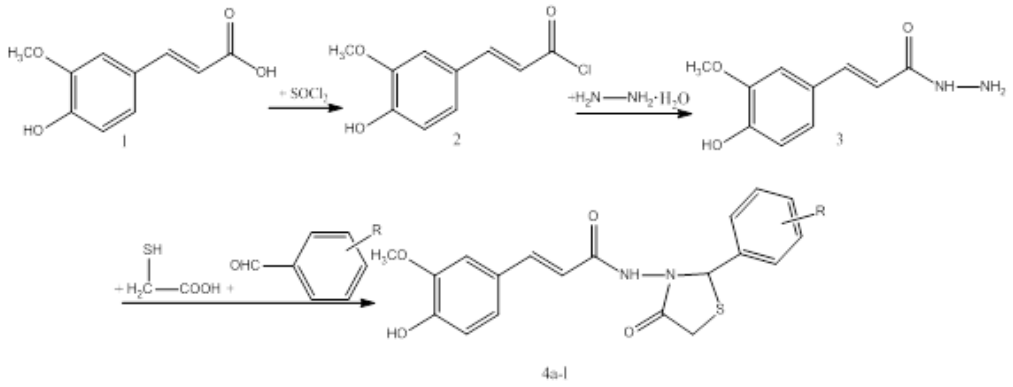

Figure 1.
The synthesis of new thiazolidin-4-ones derived from ferulic acid

General procedure for the synthesis of ferulic acid thiazolidine-4-one

To a solution of ferulic acid hydrazide (0.5 g, 2.3 mmol) in freshly distilled toluene, aromatic aldehydes (4.6 mmol) were added under inert atmosphere. The mixture was stirred for 5 min and mercaptoacetic acid 98% (0.5 mL, 6.9 mmol) was added and then it was heated at 110-120°C for 36-46 hours until completion of the reaction (TLC monitoring, using dichloromethane: methanol - 5: 0.1, v/v, UV light at 254 nm). The mixture was neutralized with saturated solution of sodium bicarbonate and extracted with ethyl acetate (2x25 mL). The organic layer was separated and washed with hydrochloric acid 1M and then with a saturated solution of sodium chloride. Finally, the organic layer was dried over $MgSO_4$ and filtered. The solvent was removed under reduced pressure. The residue was precipitated in petroleum ether.

Characterization by spectral methods. The FT-IR spectra were recorded on ABB-MB 3000 FT-IR MIRacle^{TM} Single Bounce ATR-crystal ZnSe, 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 obtained on a BrukerAvance400 MHz Spectrometer using tetramethylsilane as internal standard and deuterated dimethyl sulfoxide (DMSO-d6) as solvent. The chemical shifts were recorded in δ values (ppm).

Biological Evaluation

The *in vitro* antioxidant potential of the synthesized compounds was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging assays.

The DPPH Radical Scavenging Assay

The tested compounds were dissolved in DMSO to obtain a stock solution of 2 mg/mL (for 4k and 4l, the concentration was 0.5 mg/mL) according to the procedure from the literature [11, 12] with minor modifications. From the stock solution different volumes (50 µL, 100 µL, 150 µL, 200 µL) were measured and diluted with methanol in order to obtain 200 µL. Over the resulting samples it was added 2800 µL of methanol solution of DPPH (0.1 mM, A_{517nm}=1.0±0.05). The mixture was left for 30 min at room temperature, in the dark, and after that the absorbance was measured at 517 nm against a blank solution (methanol). The radical scavenging ability (I%) was calculted according to the following equation: $I\% = (A_0 - A_t / A_0) \times 100$. A_0 is the absorbance of DPPH methanolic solution of 0.1 mM. A_t is the absorbance of the sample at after 30 min. For each compound the effective concentration 50 (EC_{50}) was calculated by linear regression analysis and ascorbic acid (2 mg/mL) was used as positive control. All tests were performed in triplicate and the results were expressed as arithmetic average ± standard deviation (SD) [13].

The ABTS Radical Scavenging Assay

The ABTS cation radical generation was performed by treating the solution of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (7 mM) with ammonium persulfate (2.45 mM) and the mixture was left at room temperature for 16 hours in the dark. Before starting the experiment, the ABTS solution was diluted with ethanol to obtain an absorbance value of 0.700 ± 0.020 at 734 nm.

The tested compounds were dissolved in DMSO to obtain a stock solution with the concentration of 2 mg/mL (for the 4k and 4l the concentration was 0.5 mg/mL). From the stock solution different volumes (5 µL, 10 µL, 20 µL, 25 µL) were measured and then diluted with DMSO to 25 µL to which it was added 1975 µL of ABTS solution. After 6 min the absorbance at 734 nm was measured and the radical scavenging ability was calculated according to the following equation: $I\% = (A_0 - A_t / A_0) \times 100$. A_0 is the absorbance before adding the sample. A_t is the absorbance after 6 min of reaction. For each sample the effective concentration (EC_{50}) was calculated by linear regression analysis and ascorbic acid (2 mg/mL) was used as positive control. All tests were performed in triplicate and the results were expressed as arithmetic average ± standard deviation (SD) [14].

Results and Discussion

Physico-chemical and spectral characterization

The ferulic acid thiazolidine-4-ones are crystalline powders, coloured from light yellow to bright brown, very slightly soluble in dimethylformamide (DMFA), DMSO, and acetone, sparingly soluble in absolute ethanol, methanol, chloroform, dioxane and insoluble in distilled water and diethyl ether. The physico-chemical characteristics are listed in Table I.

Table I

Physico-chemical characteristics of ferulic acid thiazolidin-4-ones (4a-l)

No	R	Molecular weight	η (%)	Melting point (^{0}C)	No	R	Molecular weight	η (%)	Melting point (^{0}C)
4a	-H	370.24	15.96	102	4g	-OCH_3(2)	415.08	36.31	140-142
4b	-Cl(4)	404.06	31.63	190	4h	-OH(2)	400.45	29.01	118-120
4c	-F(4)	388.09	19.95	108-110	4i	(2,6)-diCl	386.42	50.97	213-215
4d	-Br(4)	449.32	41.24	174-176	4j	-$N(CH_3)_2$(4)	439.31	82.82	229-230
4e	-NO_2(4)	415.08	9.31	98-100	4k	(2,3)-diOH	413.14	30.94	160
4f	-NO_2(2)	415.08	36.31	140-142	4l	-OH(4) -OCH_3(3)	402.42	11.13	212

Table II

Spectral characteristics of ferulic acid thiazolidin-4-ones (4a-l)

No	FT-IR characteristic band (cm^{-1})	^{1}H-NMR signals (ppm)
4a	3225 (-NH-), 3001 (N-CH-S) 2962 (CHAr), 1690 (C=O cyclic), 1219 (C-N), 694 (C-S-C)	3.73 (s, 3H), 3.38 (s, 2H), 5.02 (d, 1H), 5.92 (d, 1H), 6.84 (d, 1H), 7.55 (d, 1H), 6.57-6.69 (m, 3H), 7.06-7.14 (m, 5H), 8.01 (s, 1H)
4b	3217 (-NH-), 2939 (N-CH-S), 1674 (C=O cyclic), 1620 (-CO-NH-), 1219 (C-N), 764 (C-Cl), 633 (C-S-C)	3.75 (s, 3H), 3.28 (s, 2H), 5.08 (d, 1H), 5.82 (d, 1H), 6.74 (d, 1H), 7.52 (d, 1H), 6.61-6.67 (m, 3H), 7.02-7.15 (m, 4H), 8.04 (s, 1H)
4c	3225 (-NH-), 2962 (CHAr), 2932 (N-CH-S), 1674 (C=O cyclic), 1157 (C-F), 640 (C-S-C)	3.72 (s, 3H), 3.31 (s, 2H), 5.04 (d, 1H), 5.86 (d, 1H), 6.71 (d, 1H), 7.51 (d, 1H), 6.59-6.65 (m, 3H), 7.04-7.14 (m, 4H), 8.02 (s, 1H)
4d	3232 (-NH-), 3024 (CHAr), 2978 (N-CH-S), 1688 (C=O cyclic), 1221 (C-N), 731 (C-S-C), <650 (C-Br)	3.69 (s, 3H), 3.29 (s, 2H), 5.01 (d, 1H), 5.79 (d, 1H), 6.89 (d, 1H), 7.54 (d, 1H), 6.61-6.67 (m, 3H), 7.10-7.21 (m, 4H), 8.12 (s, 1H)
4e	3258 (-NH-), 3078 (CHAr), 2932 (N-CH-S), 1682 (C=O cyclic), 1516, 1342 (NO_2)	3.71 (s, 3H), 3.31 (s, 2H), 5.05 (d, 1H), 5.68 (d, 1H), 6.84 (d, 1H), 7.49 (d, 1H), 6.59-6.63 (m, 3H), 7.09-7.18 (m, 4H), 8.07 (s, 1H)
4f	3107 (-NH-), 3074 (CHAr), 2920 (N-CH-S), 1684 (C=O cyclic), 1516, 1337 (NO_2), 648 (C-S-C)	3.68 (s, 3H), 3.27 (s, 2H), 5.04 (d, 1H), 5.74 (d, 1H), 6.91 (d, 1H), 7.51 (d, 1H), 6.64-6.72 (m, 3H), 7.14-7.20 (m, 4H), 8.06 (s, 1H)
4g	2964(N-CH-S), 2841 (CHAr), 1684 (C=O cyclic), 667 (C-S-C)	3.73 (s, 6H), 3.34 (s, 2H), 5.01 (d, 1H), 5.81 (d, 1H), 6.86 (d, 1H), 7.52 (d, 1H), 6.57-6.70 (m, 7H), 8.06 (s, 1H)
4h	3045 (-NH-), 2974(CHAr), 2922 (N-CH-S), 1620 (C=O cyclic), 683 (C-S-C)	3.71 (s, 3H), 3.31 (s, 2H), 5.04 (d, 2H), 5.76 (d, 1H), 6.81 (d, 1H), 7.54 (d, 1H), 6.52-6.76 (m, 7H), 8.02 (s, 1H)
4i	2986 (N-CH-S), 2955 (CHAr), 1680 (C=O cyclic), 779 (C-Cl), 704 (C-S-C)	3.67 (s, 3H), 3.31 (s, 2H), 5.01 (d, 1H), 5.75 (d, 1H), 6.84 (d, 1H), 7.52 (d, 1H), 6.51-6.72 (m, 6H), 8.05 (s, 1H)
4j	2918 (N-CH-S), 2854 (CHAr), 1691 (C=O cyclic), 690 (C-S-C)	2.85 (s, 6H) 3.71 (s, 3H), 3.28 (s, 2H), 5.04 (d, 1H), 5.71 (d, 1H), 6.81 (d, 1H), 7.49 (d, 1H), 6.54-6.78 (m, 7H), 8.02 (s, 1H)
4k	3483 (OH), 3408 (-NH-), 2951 (CHAr), 2924 (N-CH-S), 1624 (C=O cyclic), 1223 (C-N), 658 (C-S-C)	3.71 (s, 3H), 3.35 (s, 2H), 5.04 (d, 3H), 5.89 (d, 1H), 6.82 (d, 1H), 7.53 (d, 1H), 6.57-6.78 (m, 6H), 8.01 (s, 1H)
4l	3022 (-NH-), 2957 (CHAr), 2926 (N-CH-S), 1676 (C=O cyclic), 1624 (CO-NH-) 1271 (C-N), 683 (C-S-C)	3.73 (s, 6H), 3.32 (s, 2H), 5.01 (d, 2H), 5.82 (d, 1H), 6.87 (d, 1H), 7.48 (d, 1H), 6.51-6.81 (m, 6H), 8.03 (s, 1H)

In the FT-IR spectra the appearance of the stretching band of the C=O of thiazolidine-4-one at 1620-1691 cm^{-1}, together with the characteristic absorption band of C-S at 633-694 cm^{-1} confirm the success of the cyclization reaction and the formation of thiazolidine system. The characteristic bands of the aromatic ring appeared in the range of 3078-2841 cm^{-1}, and the phenyl ring substituents were observed at 1157 cm^{-1} (C-F), 764 cm^{-1} (C-Cl), 1516, 1337 cm^{-1} (NO_2), 3483 cm^{-1} (OH), <650 $cm^{-1.}$ (C-Br). The formation of the thiazolidine-4-one system has also been proved by the NMR data. In the ^{1}H-NMR spectra the proton of CH (SCHN) resonates as a singlet, between 5.92-5.68 ppm and the protons of the methylene group (-CH_2-S) appear as doublet between 3.38-3.27 ppm. The spectral characteristics are listed in Table II.

The antioxidant activity

The DPPH Radical Scavenging Assay. The method is based on the spectrophotometric determination of the ability of inactivating the purple stable free radical DPPH (2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl) by reduction to 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazine) yellow under the action of compounds with antioxidant effects. From the results obtained (Table III) it was observed that the most favourable influence was exerted by 2-OH-3-OH, 4-OH-3-OCH_3 radicals, and the corresponding compounds 4k and 4l being 8.84 and 3.17 times respectively more active than ferulic acid (FA). Moreover the compound 4k was 1.76 times more active than ascorbic acid (AA) used as positive control. A good antioxidant effect was showed also by the compounds 4c (R=4-F), 4e (R=4-NO_2) and 4i (R=2,6-diCl), which presented an effect comparable with that of ferulic acid.

Table III

The DPPH radical scavenging ability (EC_{50}, µg/mL) of tested compounds (4a-l)

No	EC_{50} µg/mL	No	EC_{50} µg/mL	No	EC_{50} µg/mL
FA	31.13 ± 0.07	4e	24.56 ± 0.17	4i	29.98 ± 0.22
4a	29.24 ± 0.60	4f	57.75 ± 0.20	4j	32.85 ± 0.38
4b	53.95 ± 0.20	4g	64.28 ± 0.14	4k	3.52 ± 0.01
4c	24.25 ± 0.35	4h	126.57 ± 0.48	4l	9.79 ± 0.26
4d	65.08 ± 0.20	AA	6.21± 0,023		

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

The ABTS Radical Scavenging Assay. The antioxidants reduce the radical cation $ABTS^+$ by an electron transfer mechanism with colour variation from blue to yellow. From the results obtained (Table IV) it was observed that the thiazolidin-4-one synthesized by the cyclization of ferulic acid hydrazide with benzaldehyde and thioglycolic acid (4a) is 1.25 times more active than ferulic acid. Related to the influence of the radical which substituted the aromatic ring it was found that the most favourable influence was exerted by 2,3-OH (4k), 4-OH-3-OCH_3, the corresponding compounds 4k and 4l being 8.16 and 5.21 times respectively more active than ferrulic acid (FA). Moreover the compounds were 4 times (4k) and 2.5 times (4l) more active than ascorbic acid (AA) used as positive control. A good antioxidant effect was also showed by the compounds 4i (R = 2,6-diCl), 4c (R = -F) and 4e (R = -NO_2). These compounds were 1.7, 1.57 and 1.56 times respectively more active than ferulic acid and their activity was comparable with ascorbic acid (AA).

Table IV

The ABTS radical scavenging ability (EC_{50}, µg/mL) of tested compounds (4a-l)

No	EC_{50} µg/mL	No	EC_{50} µg/mL	No	EC_{50} µg/mL
FA	10.37 ± 0.02	4e	6.62 ± 0.07	4i	6.08 ± 0.02
4a	8.29 ± 0.08	4f	14.05 ± 0.04	4j	11.41 ± 0.02
4b	11.04 ± 0.08	4g	17.50 ± 0.06	4k	1.27 ± 0,01
4c	6.58 ± 0.07	4h	12.29 ± 0.03	4l	1.99 ± 0,01
4d	12.07 ± 0.07	AA	5.11 ± 0.09		

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

Conclusions

New thiazolidin-4-ones of ferulic acid have been synthesized and optimal conditions of reaction were established. The synthesized compounds have been characterized by their physical constants (melting point, yield, molecular formula, molecular weight, solubility in different organic solvents) and the chemical structure was proved using FT-IR and ^{1}H-NMR spectroscopy. It was also assessed the antioxidant potential using *in vitro* methods. Some of the tested compounds showed appreciable antioxidant activity in reference to ferulic acid, which confirms the favourable influence of structural modulation on the antioxidant effects of ferulic acid.

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