SYNTHESIS, CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF SOME NEW THIAZOLIDIN-4-ONE DERIVATIVES

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SYNTHESIS, CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF SOME NEW THIAZOLIDIN-4-ONE DERIVATIVES (Abstract): **Aim:** To design new thiazolidin-4-ones derivatives and to evaluate their potential antioxidant effects using *in vitro* methods. **Material and methods:** New ethyl esters of the 2-(R-phenyl)-4-oxo-thiazolidin-3-yl propionic acid were synthesized using "one step reaction" between different aromatic aldehydes, thioglycolic acid and beta-alanine ethyl ester hydrochloride. The antioxidant potential of the synthesized compounds was evaluated using the DPPH radical scavenging assay and phosphomolybdenum method. **Results:** Eight thiazolidine-4-one derivatives were obtained in good yields and high purity. The structure of the synthesized compounds was confirmed using IR spectroscopy. The evaluation of antioxidant activity showed that 2-[(4-NO_2)-phenyl]-4-oxo-thiazolidin-3-yl propionic acid ethyl ester (compound 16) was the most active compound. For this derivative the DPPH radical scavenger activity (I% = 91.63%±0.77) and the total antioxidant capacity (absorbance = 1.0691±0.0763) were similar with that of ascorbic acid used as standard antioxidant. **Conclusions:** The antioxidant activity of the thiazolidine-4-one derivatives depends on the nature of the phenyl ring substituents, the NO_2 and OH radicals having the most significant influence. **Keywords:** SYNTHESIS, THIAZOLIDIN-4-ONE, ANTIOXIDANT ACTIVITY.

The concern to design thiazolidine derivatives started in 1928 when penicillin was discovered, and since then the development of new derivatives kept increasing. So far, the thiazolidin-4-ones derivatives had been found to posses various biological actions: anti-HIV1 (1, 2), antibacterial (3, 4), antitubercular (5), antioxidant (6, 7), anti-inflammatory (3, 8) and antitumoral (7, 9, 10).

On the other hand, it is known that oxidative stress is involved in the occurrence and development of many diseases, there are more than 200 diseases caused by oxidative stress and 39 deadly diseases for which there is evidence validating their positive link with oxidative stress. Antioxidants inhibit the oxidative damage induced by radicals and reactive oxygen species (ROS) (11). Thus, the therapy with antioxidants has gained ground in the last years.

Furthermore, commercial availability of

a drug is depending on the cost of production line. A simple and efficient method, a small number of synthetic steps or selectivity in a reaction of many components is desirable for obtaining the drug.

The aim of this study was the synthesis of new thiazolidin-4-one derivatives using an one- step reaction ("domino" reaction) and evaluation of their potential antioxidant effects.

MATERIAL AND METHODS

Reagents. Aromatic aldehyde (4-bromo/4-fluoro/4-chloro/2-methoxy/4-methoxy/4-nitro/2-hydroxi/4-hydroxi-benzaldehyde), thioglycolic acid, beta-alanine ethyl ester hydrochloride, *N,N*-diisopropyl-ethylamine (DIEA), and standard reagents for antioxidant assays were purchased from Sigma Aldrich or Flucka. The toluene was freshly distilled and other solvents were used without further purification. TLC silica gel 60 F_{254} from Merck was used to monitor the reactions.

Methods

Chemistry. The aromatic aldehydes (**1-8**), beta-alanine ethyl ester hydrochloride (**9**) and thioglycolic acid (**10**) were mixed in the presence of *N,N*-diisopropyl-ethylamine (DIEA) used as catalyst. The reaction was carried out by adapting similar methods used for synthesis of other thiazolidine derivatives (12).

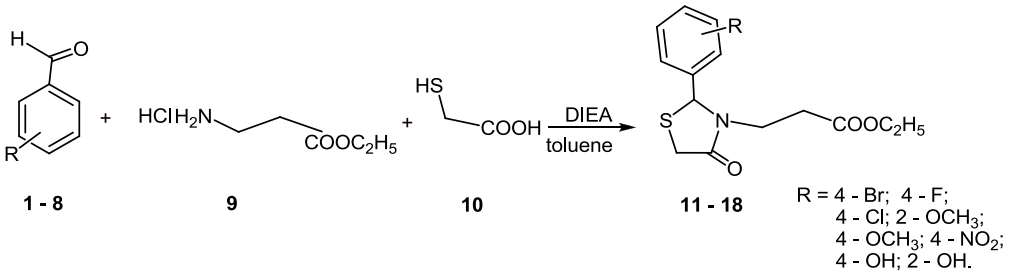


Fig. 1. The synthesis of new ethyl esters of the 2-(R-phenyl)-4-oxo-thiazolidin-3-yl propionic acid

General procedure for synthesis of thiazolidin-4-one derivatives. To beta-alanine ethyl ester hydrochloride (0.01 mol) in 10 ml toluene the aromatic aldehyde (0.015 mol) was added. The mixture was stirred for 5 min and then mercaptoacetic acid 98% (0.02 mol, 1.25 mL) was added. The mixture was stirred again for 5 min and after that *N,N*-diisopropyl-ethylamine (DIEA) (0.013 mol, 2.26 mL) was added and then the mixture was heated to 110-115°C under reflux for 24-30 h under argon. The water was removed by colleting it in a Dien Stark apparatus. The reaction was monitored by TLC using as eluent ethyl-acetate/petroleum ether = 4:6.

The reaction mixture was neutralized with saturated solution of sodium bicarbonate and the organic phase was extracted using ethyl acetate (2 x 25 mL). The organic layer was successively washed with 1M HCl solution and saturated sodium chloride solution, dried over anhydrous $MgSO_4$ and finally filtrated. The filtrate was evaporated and the residue was passed through a silica gel column.

Physico-chemical characterization

Fourier transform infrared (FTIR) spec-

tra were recorded on a Nicolet iS50 FT-IR Spectrometer. All spectra were recorded in the 4000-600 cm^{-1} range at resolution of 4 cm^{-1} over 16 scans. Spectroscopy data were processed with an Omnic Spectra Software. The value of logP (logarithm of partition coefficient between n-octanol and water) as a measure of compound hydrophilicity was calculated using Osiris Property Explorer software (clogP)

Antioxidant assays

The total antioxidant capacity of the tested compounds was evaluated using the phosphomolybdenum method according to the standard procedure of Pietro *et al.* (1999) with some modifications. Briefly, the sample solution (300 µL, 5 mg/mL in methanol) was mixed with 3 mL reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM amonium molybdate). The samples were incubated at 95°C for 90 min. After the mixture has cooled to room temperature, the absorbance of samples was measured at 695 nm against a blank (300 µL methanol mixed with 3 mL reagent solution) using the GBC-Cintra 2020 UV-VIS Spectrophotometer. Ascorbic acid was used as standard antioxidant.

The DPPH radical scavenging activity. DPPH is a stable nitrogen-centered free radical that is scavenged by antioxidants, changing its color from violet to yellow upon reduction by either the process of hydrogen or electron donation. The sample solution (300 µL, 10 mg/mL in methanol) was added to 2.7 mL solution of DPPH in methanol (dilution 1% from a solution of 0.043%).

The mixture was left for 30 min in the dark and then absorbance was measured at 515 nm against a blank using the UV-VIS spectrophotometer. Ascorbic acid was used as standard antioxidant.

The scavenging capacity was calculated according to the following equation:

$I\% = (Abs_{t=0} - Abs_{t=30min}/Abs_{t=0}) \times 100$

where:

$Abs_{t=30min}$ is the absorbance of the tested compound after 30 minutes.

$Abs_{t=0}$ is absorbance of DPPH methanol solution without sample.

Statistical analysis. The antioxidant assays were carried out in triplicate. Data were analyzed by the analysis of variance (ANOVA) ($p < 0.05$) and were expressed as means ± SD.

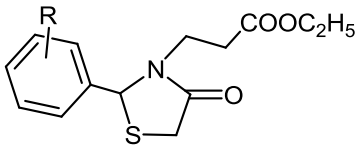
RESULTS AND DISCUSSION

Chemistry. The physical characteristics of the compounds are presented in Table I. The compounds looked like colorless or yellow liquids, excepted for the compound (17) that was solid. The value of *clogP* calculated using a free software showed a high lipophilicity (value of the *clogP* is more than 2.5) for thiazolidin-4-one compounds substituted on aromatic ring with 4-Br (*clogP* = 2.76), 4-Cl (*clogP* = 2.67) and 4-NO_2 (*clogP* = 2.60).

The chemical structure of the compounds was confirmed using FT-IR spectroscopy (tab. II). In the IR spectra the absorption band of CO from ester group appeared in the range of 1726-1728.87 cm^{-1}. The thiazolidine ring was identified by the bands characteristic of CO group (1673-1676 cm^{-1}) and C-S bond (622-644.5 cm^{-1}). The characteristic bands of aromatic ring appeared in the range of 2979-2981 cm^{-1} and the phenyl ring substituents appeared at: 1155.62 cm^{-1} (C-F), 767.93 (C-Cl), 1520.4 and 1344.87 cm^{-1} (NO_2), 3270.59 (O-H), 3233.78 (O-H). The C-Br bond had a absorption less than 650 cm^{-1}.

TABLE I

Physical characteristics of thiazolidin-4-one derivatives



Comp.	R	Physical characterization					clogP
		Molecular Formula	Molar Mass	Aspect	Yield (%)	Rf*	
11	4-Br	$C_{14}H_{16}BrNO_3S$	358.25	light yellow liquid	67%	0.64	2.76
12	4-F	$C_{14}H_{16}FNO_3S$	297.08	colorless liquid	42%	0.62	2.12
13	4-Cl	$C_{14}H_{16}ClNO_3S$	313.80	colorless liquid	46%	0.61	2.67
14	4-OCH_3	$C_{15}H_{19}NO_4S$	309.38	light yellow liquid	71%	0.50	1.95
15	2-OCH_3	$C_{15}H_{19}NO_4S$	309.38	yellow liquid	82%	0.51	1.95
16	4-NO_2	$C_{14}H_{16}N_2O_5S$	324.35	yellow liquid	54%	0.47	2.60
17	4-OH	$C_{14}H_{17}NO_4S$	295.35	white solid**	24%	0.2	1.76
18	2-OH	$C_{14}H_{17}NO_4S$	295.35	brown yellow liquid	37%	0.3	1.76

Rf*: ethylacetate: petroleum ether = 4:6; white solid**: m.p = 98^{o}C

TABLE II

IR Spectral characteristic bands of thiazolidin-4-one derivatives

Comp.	R	IR characteristic band (cm^{-1})				
		Ar-H	C=O ester	C=O Ring	C-S	R
11	4-Br	2979.88	1727.42	1674.19	625.07	<650 (Br)
12	4-F	2981.63	1727.38	1674.28	636.25	1155.62 (F)
13	4-Cl	2980.48	1727.40	1674.06	622.07	767.93 (Cl)
14	2-OCH_3	2979.74	1727.16	1674.34	644.94	2838.23 (C-H)
15	4-OCH_3	2979.52	1728.11	1673.79	628.68	2837.11 (C-H)
16	4-NO_2	2981.11	1726.37	1675.79	631.98	1520.4; 1344.87 (NO_2)
17	4-OH	2980.25	1727.87	1650.39	632.68	3270.59 (OH)
18	2-OH	2980.80	1728.72	1645.82	640.57	3233.78 (OH)

Antioxidant assays.

The total antioxidant capacity. The assay for total antioxidant capacity was based on the reduction of Mo^{+6} to Mo^{+5} by the tested

compounds followed by formation of a green phosphate/Mo^{+5} complex at acid pH. The results of the tested compounds are listed in table III. The most active compound was thiazolidin-4-one derivative with NO_2 on the phenyl ring (compound 16), for which the absorbance (1.0691±0.0763) was similar with that of ascorbic acid used as reference (1.00±0.4052). The compound (11) with Br as substituent on aromatic ring also had important activity (0.8467±0.0406), the value of the absorbance being comparable with that of ascorbic acid.

The DPPH radical scavenger activity. The results of the DPPH radical scavenger assay are presented in fig. 2. The most active compound was compound 16 with NO_2 as substituent on aromatic ring for which the inhibition (91.63%±0.77) was very close to that of ascorbic acid (AA) (99.01 ± 0.32). Compounds 17 and 18 with OH radical on aromatic ring also showed important scavenger activity, the inhibition exceeding 60%.

TABLE III

Absorbance of the tested thiazolidin-4-one derivatives (5 mg/ml)

Sample	R	Absorbance	Sample	R	Absorbance
11	4-Br	0.8467±0.0406	15	4-OCH_3	0.4622±0.0221
12	4-F	0.5408±0.0171	16	4-NO_2	1.0691±0.0763
13	4-Cl	0.3980±0.0124	17	4-OH	0.4955±0.0119
14	2-OCH_3	0.6006±0.0519	18	2-OH	0.47283±0.003
Ascorbic acid (AA)*			1.00±0.4052		

*Ascorbic acid 0.125mg/mL

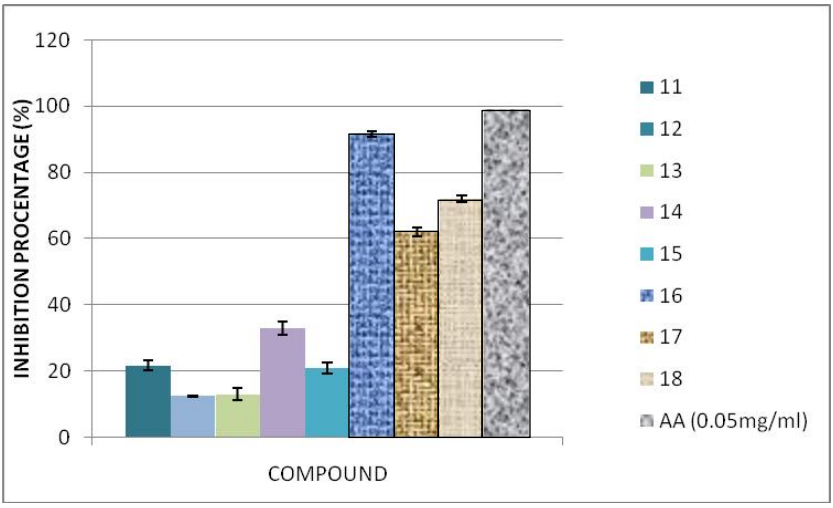


Fig. 2. Antioxidant activity – DPPH scavenger radical

CONCLUSIONS

Some new 1,3-thiazolidin-4-one derivatives have been synthesized. The synthesized compounds have been physically characterized (yield, molecular formula, molecular weight, solubility in different organic solvents, clogP) and their chemical structure was confirmed by FT-IR spectroscopy. The

antioxidant potential of the synthesized compounds has been evaluated using the DPPH radical scavenger assay and phosphomolybdenum method. The results suggest that the most active compound is 2-[(4-NO_2)-phenyl]-4-oxo-thiazolidin-3-yl propionic acid ethyl ester (compound 16) the DPPH radical scavenger activity and total antioxidant capacity of which was comparable to that of ascorbic acid.

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