SYNTHESIS AND EVALUATION OF ANTIOXIDANT ACTIVITY OF SOME NEW BENZYLIDENE-THIAZOLIDINE-XANTHINE DERIVATIVES

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SYNTHESIS AND EVALUATION OF ANTIOXIDANT ACTIVITY OF SOME NEW BENZYLIDENE-THIAZOLIDINE-XANTHINE DERIVATIVES (Abstract): The International Diabetes Federation reported that 246 million adults worldwide had diabetes mellitus and the prevalence of this syndrome was expected to increase continuously. **Aim:** To design new compound with potential antidiabetic and antioxidant activity. **Material and methods:** New benzylidene-thiazolidine derivatives (BT2a-2e) were obtained by condensation of xanthine-thiazolidine-4-one (TZ-4-one) with aromatic aldehydes. The synthesized compounds were characterized by spectral method (IR, ¹H-NMR, ¹³C-NMR) and their antioxidant potential has been also evaluated. **Results:** The synthesized compounds have important antioxidant effects as compared to xanthine-thiazolidine derivatives. The most active compounds were those obtained by condensation with 4-dimethylaminobenzaldehyde (BT2c) and 4-nitro-benzaldehyde (BT2e). **Conclusions:** The chemical modulations performed on the structure of TZD-4-one have a good influence on their antioxidant potential. **Keywords:** XANTHINE, THIAZOLIDINE, SPECTRAL METHODS, ANTIOXIDANT

Type 2 diabetes mellitus (T2DM) is considered to be the „epidemic of the 21st century” and the discovery of new therapies is a challenge for researchers. An inadequate glycemic control can be the leading cause of cardiovascular disorders, blindness, renal failure and amputations (1).

Due to the complex nature of this disease and decreased beta cell function, the management of glycemic control is difficult. There are currently used distinct classes of hypoglycemic agents: biguanides, sulfonylureas, meglitinides, thiazolidinediones, α-glucosidase inhibitors. The disadvantages of this therapy are the multiples side effects (weight gain, lactic acidosis, flatulence, oedema, fracture, liver and renal failure, pancreatitis) and more importantly the difficulty to obtain a good glycemic control. The new goal in T2DM management is the development of incretin modulators and dipeptidyl peptidase IV inhibitors (DPP IV).

DPP IV inhibitors are of considerable
interest to the pharmaceutical industry and intense researches in this area have resulted in the release to the market of such drugs as: sitagliptin, saxagliptin, linagliptin, alogliptin, vildagliptin (2). Linagliptin is a xanthine-based orally administered long acting drug, which at a 5 mg dose inhibits DPP IV activity more than 80% (3).

Hyperlipidemia and free radicals are among the most important factors involved in the pathogenesis of diabetes. Persistent hyperglycemia causes increased production of free radicals, especially reactive oxygen species resulting from glucose auto-oxidation and protein glycosylation (4).

This study presents the synthesis, physico-chemical and spectral characterization, and evaluation of the antioxidant activity of new xanthine scaffold benzylidene-thiazolidine-4-ones, in order to obtain new compounds with potential antidiabetic and antioxidant action.

**MATERIAL AND METHODS**

4-brom/4-fluor/4-N-dimethylamino/2-nitro-benzaldehyde; vitamin C; vitamin E; DPPH; ABTS; potassium persulfate; monosodium and disodium phosphate; ammonium molybdate; potassium ferricyanide; trichloracetic acid; iron chloride. $^1$H-NMR and $^{13}$C-

NMR were recorded in DMSO-\textit{d}_6 with a BrukerAvance 300 MHz instrument. The chemical shift was expressed in ppm as a function of tetramethysilane (TMS) as internal standard. FTIR spectra were recorded on a Biorad FT-IR- FTS 575C spectrophotometer. All melting points were determined on Buchi B-540 Melting Point apparatus.

**Chemistry**

The compounds were obtained through condensation of 2-\{2-(1,3-dimethylxanthin-7-yl)acetyl\}hydrazono\}-3-(4-brom) phenylthiazolidin-4-one (TZ-4-one, 1) with different substituted aromatic aldehydes, by adapting similar methods described in the literature (5) (Scheme 1).

**General procedure for BT 2a-2e synthesis**

2-\{2-(1,3-dimethylxantin-7-il)acetyl\}hydrazono\}-3-(4-brom)phenylthiazolidin-4-one (4g, 0.0079 mol) was dissolved in 100 ml dioxane. To this solution aromatic aldehyde (0.0079mol) and piperidine (0.00395) were added as catalyst and maintained under reflux for 6 h. The solvent was removed under reduced pressure and the final compound was precipitated in cold water. The solid product separated was filtered, washed with excess of cold water, dried and recrystallized from dioxane.

**Scheme 1.** Synthesis of xanthine scaffold benzylidene-thiazolidine-4-one derivatives
2-{[2-{[1,3-dimethylxanthine-7-yl]acetyl][hydrazono]-5-(4-florobenzylidene)-3-(4-brom-phenyl)-thiazolidine-4-one (BT2a). Yellow powder; yield 69.8%; m.p. 270-271°C; FT-IR (cm⁻¹): 1642.1 (-C=N), 1228.4 (-C-N), 1594.8 (-C=O amide), 1491.6, 1425.1 (-N-CH₂), 3246.5 (-NH-), 1699.9 (-C=O thiazolidine moiety), 760.7 (C-S-C), 497.5 (-C-Br), 973.8 (-C-F); ¹H-NMR δ/ppm (300 MHz, DMSO): 8.2 (s, 1H, CH=N-); 7.5-7.51 (m, 4H, benzylidene); 7.5-7.4 (m, 4H, phenyl); 5.8 (s, 2H, -CH₂-C=O); 3.4-3.2 (s, 6H, 2CH₃); ¹³C-NMR δ/ppm (300 MHz, DMSO): 165.2 (C₁₂); 154.7, 150.9 (C₂, C₆); 148.4 (C₄); 139.6, 131.7, 119.2 (C₂₀, C₂₄, C₂₃); 113.2 (C₁₁); 105.7 (C₃); 44.2 (C₈); 29.4, 27.5 (C₁, C₃).

2-{[2-[1,3-dimethylxanthine-7-yl]acetyl][hydrazono]-5-(4-brombenzylidene)-3-(4-bromphenyl)-thiazolidine-4-one (BT2b). Yellow powder; yield 68.4%; m.p. 273-275°C; FT-IR (cm⁻¹): 1643.05 (-C=N), 1228.43 (-C-N), 1594.84 (-C=O amide), 1497.56, 1426.1 (-N-CH₂), 3247.5 (-NH-), 1699.94 (-C=O thiazolidine moiety), 760.78 (C-S-C), 497.54 (-C-Br); ¹H-NMR δ/ppm (300 MHz, DMSO): 8.28 (s,1H, -CH=N-); 7.57-7.51 (m, 4H, benzylidene); 7.54-7.48 (m, 4H, phenyl); 5.8 (s, 2H, -CH₂-C=O); 3.44, 3.23 (s, 6H, 2CH₃); ¹³C-NMR δ/ppm (300 MHz, DMSO): 165.27 (C₁₂); 154.74, 150.95 (C₂, C₆); 148.42 (C₄); 139.63, 131.76, 119.29 (C₂₀, C₂₄, C₂₃); 113.22

2-{[2-{[1,3-dimethylxanthine-7-yl]acetyl][hydrazono]-5-(4-N-dimethylaminobenzylidene)-3-(4-bromphenyl)-thiazolidine-4-one (BT2c).Orange powder; yield 63.4%; m.p. 264-265°C; FT-IR (cm⁻¹): 1643.1 (-C=N), 1228.4 (-C-N), 1594.8 (-C=O amide), 1468.5, 1426.1 (-N-CH₂), 3247.5 (-NH-), 1699.9 (-C=O thiazolidine moiety), 760.7 (C-S-C), 497.5 (-C-Br), 3107 [-N(CH₃)₂]; ¹H-NMR δ/ppm (300 MHz, DMSO): 8.28 (s, 1H, -CH=N-); 7.58-6.9 (d, 4H, 4CH benzylidene); 7.5-7.35 (d, 4H, phenyl); 5.79 (s, 2H, -CH₂-C=O); 3.44-3.23 (s, 6H, 2CH₃); 3.05 (s, 6H, 2CH₃benzylidene).¹³C-NMR δ/ppm (300 MHz, DMSO): 165.6 (C₁₂); 154.4, 150.9 (C₂, C₆); 148.4 (C₄); 140.3, 129.05, 121.9 (C₂₀, C₂₄, C₂₃); 117.37 (C₁₁); 105.77 (C₃); 44.21 (C₈); 40.32 (C₂₆ and C₂₇-N(CH₃)₂); 29.47, 27.51 (C₁, C₃).

2-{[2-{[1,3-dimethylxanthine-7-yl]acetyl][hydrazono]-5-benzylidene-3-(4-bromphenyl) -thiazolidine-4-one (BT2d).Yellow powder; yield 67%; m.p. 245-247°C; FT-IR (cm⁻¹): 1642.1 (-C=N), 1228.4 (-C-N), 1592.8 (-C=O amide), 1464.6-1423.1 (-N-CH₂), 3245.5 (-NH-), 1697.9 (-C=O thiazolidine moiety), 758.7 (C-S-C), 497.5 (-C-Br); ¹H-NMR δ/ppm (300 MHz, DMSO): 8.2 (s,1H, -CH=N-); 7.5-7.4-7.3 (m, 5H, 5CH benzylidene); 7.5-7.4 (m, 4H, phenyl); 5.8 (s, 2H, -CH₂-C=O); 3.4-3.3 (s, 6H, 2CH₃); ¹³C-NMR δ/ppm (300 MHz, DMSO): 165.2 (C₁₂); 154.7, 150.9 (C₂, C₆); 148.42 (C₄); 139.6, 131.7, 119.2 (C₂₀, C₂₄, C₂₃); 113.2 (C₁₁); 105.7 (C₃); 44.2 (C₈); 29.4, 27.5 (C₁, C₃).

2-{[2-{[1,3-dimethylxanthine-7-yl]acetyl][hydrazono]-5-(2-nitrobenzylidene)-3-(4-bromphenyl)-thiazolidine-4-one (BT2e).Yellow powder; yield 77%; m.p. 253-255°C; FT-IR (cm⁻¹): 1643.05 (-C=N), 1228.43 (-C-N), 1594.84 (-C=O amide), 1497.56, 1426.1 (-N-CH₂), 3247.5 (-NH-), 1699.94 (-C=O thiazolidine moiety), 760.78 (C-S-C), 497.54 (-C-Br); ¹H-NMR δ/ppm (300 MHz, DMSO): 8.28 (s, 1H, CH=N-); 8.06-7.5 (s, 4H, benzylidene); 7.5-7.4 (m, 4H, phenyl); 5.8 (s, 2H, -CH₂-C=O); 3.4-3.2 (s, 6H, 2CH₃); ¹³C-NMR δ/ppm (300 MHz, DMSO): 165.26 (C₁₂); 154.74, 150.95 (C₂, C₆); 148.42 (C₁₈); 139.63, 131.76, 119.29 (C₂₀, C₂₄, C₂₃); 113.22 (C₁₁); 105.76 (C₃); 44.19 (C₈); 29.47, 27.5 (C₁, C₃).
EVALUATION OF ANTIOXIDANT ACTIVITY

Evaluation of antioxidant activity through phosphomolybdenic method. Different sample concentrations were obtained by dilution of stock solutions (5 mg/ml). An aliquot of sample solution (0.2 ml) was mixed with the reagent solution (2 ml, 28 mM sodium phosphate; 4 mM ammonium molybdate; 0.6 M sulphuric acid). The samples were incubated at 95°C for 90 min and then centrifuged; absorbance was measured at 695 nm. The antioxidant activity of the samples was compared with that of vitamin C (2 mM) (6).

Reducing power assay. The compounds possessing electron donating groups reduce Fe³⁺ to Fe²⁺ and with iron chloride form Berlin blue with a maximum absorbance at 700 nm. To 1 ml of sample solutions obtained from stock solution (5 mg/ml), 1 ml buffer phosphate (pH 6.6) was added. The mixture was shaken vigorously and 1 ml potassium ferricyanide 1% was added. After incubation 20 min at 50°C 1 ml trichloracetic acid 10% was added and then centrifuged at 4500 rpm for 15 minutes. To 1 ml of supernatant, 1 ml distilled water and 0.2 ml ferric chloride 0.1% were added. After 5 min the absorbance was measured at 700 nm. Vitamin C was used as reference (7).

Scavenging effect of antioxidant activity on DPPH radical. DPPH is a stable free radical that can accept hydrogen atoms. The reduced form of DPPH is 2,2'-diphenyl-1-picrylhydrazine of yellow color. Various concentrations of the test compounds in 200 μl DMSO were added over 2500 μl solution of DPPH radical in methanol (0.1 mM). The mixture was allowed to stand for 1 hour in the dark and then absorbance was measured at 517 nm against a blank using the UV-VIS spectrophotometer. The radical scavenging capacity was calculated according to the following equation: \( I\% = \left( \frac{A_{t=0} - A_{t=60\ min}}{A_{t=0}} \right) \times 100 \). \( A_{t=60\ min} \) is the test compound absorbance after 1 hour. \( A_{t=0} \) is absorbance of 0.1 mM DPPH methanol solution at 517 nm. Vitamin E was used as reference (8).

Scavenging effect of antioxidant activity on ABTS radical. The ABTS⁺ radicals were produced by reacting ABTS (7 mM) (2,2’-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) with ammonium persulphate (2.45 mM) for 16 hours in the dark as described in literature (9). This stock solution was diluted with ethanol to obtain a solution with absorbance of 0.7 ± 0.02 at 734 nm. The tested compounds were dissolved in DMSO-ethanol (5 mg/ml) and 0.02 ml of different concentrations was added to 1.980 ml ABTS solution. After 6 min the absorbance was measured and the radical scavenging capacity was calculated according to the following equation:

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I\% = 100 \times \left( \frac{A_{\text{initial}} - A_{\text{final}}}{A_{\text{initial}}} \right),
\]

where the \( A_{\text{initial}} \) = absorbance before adding the sample, \( A_{\text{final}} \) = absorbance after 6 min of reaction. Vitamin E was used as referee.

RESULTS AND DISCUSSION

The conclusions referring to antioxidant activity pointed out that the chemical modifications on the structure of 2-{2-[2-(1,3-dimethylxanthine-7-yl)acethyl] hydrazono}-3-(4-brom)phenylthiazolidine-4-one (TZD-4-one) through their condensation with different aromatic aldehydes (benzaldehyde, 4-brom/4-fluor/4-dimethylamino/2-nitrobenzaldehyde) have a good influence on the antioxidant potential, all tested compound being more active than TZD-4-one. As to the antioxidant activity determined by phosphomolybdenic reagent reduction method, it has been noticed that the
most active compounds are BT2c (EC50=29.37) and BT2e (EC50=33.13), obtained through condensation with 4-dimethylaminobenzaldehyde and 2-nitrobenzaldehyde, being more active than TZD-4-one (EC50=136.9) by up to 4.7 and 4.2 times, respectively (fig. 1). These compounds were proved to be the most active of all, reducing power included; BT2c (EC50=23.13) was 6 times and BT2e (EC50=41.6) 3.3 times more active than TZD-4-one (EC50=137.857) (fig. 2).

The study of radical scavenging action determined by the reduction of DPPH (fig. 3) and ABTS radicals (fig. 4) showed that compound BT2c radicals was the most active of them. Its scavenging activity was 3.7 times (EC50=38.13, DPPH method), and 3.3 times (EC50=21.17, ABTS method), respectively more intense than that of TZD-4-one. An appreciable antioxidant activity compared with TZD-4-one was also found for BT2e (EC50=46.53 and 33.2, respectively).

**CONCLUSIONS**

New benzylidene-thiazolidine derivatives with xanthine moiety have been synthesized, and physico-chemically characterized (melting point, yield, solubility in different organic solvents). Chemical structure was confirmed by spectral methods...
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(IR, $^1$H-NMR, $^{13}$C-NMR). The antioxidant potential of the synthesized compounds was evaluated, their antioxidant activity, reducing capacity and scavenging activity (by DPPH and ABTS methods) being determined. The most active proved to be the compounds resulted from condensation with 4-N-dimethylaminobenzaldehyde (BT2c) and 2-nitrobenzaldehyde (BT2e).

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