ORIGINAL ARTICLE

STUDIES ON XANTHINE DERIVATIVES (II). SYNTHESIS AND ANTIOXIDANT EFFECTS OF SOME HYDRAZONES WITH XANTHINE STRUCTURE

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Abstract

A series of thirteen hydrazones with xanthine structure (derivatives of theophylline) have been synthesized and characterized in terms of physical and chemical properties. Their structure was proved using spectral methods (infrared – IR, proton nuclear magnetic resonance – ^{1}H-NMR, carbon-13 nuclear magnetic resonance – ^{13}C-NMR, high resolution mass spectroscopy – HRMS). The antioxidant effects of the synthesized compounds were evaluated using *in vitro* assays: DPPH (2,2-Diphenyl-1-picrylhydrazyl**)** and ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging ability and total antioxidant capacity. The results showed that the chemical modulation of theophylline was associated with improving of the antioxidant effects of the parent compound, generally. The most active compound was hydrazone **c**, which has 2-OH as substituent on the aromatic ring, its ABTS scavenging activity being comparable with ascorbic acid. The substitution of the aromatic ring with 3/4-OH, 4-NO_2 and 4-CN was also associated with improved antioxidant effects, the corresponding derivatives having appreciable DPPH and ABTS scavenging effects and total antioxidant capacity.

Rezumat

O serie de 13 hidrazone cu structură xantinică (derivați de teofilină) au fost sintetizate și caracterizate din punct de vedere fizico-chimic. Structura lor a fost confirmată utilizând metode spectrale (infraroșu – IR, rezonanță magnetică nucleară a nucleilor de hidrogen – ^{1}H-RMN, rezonanță magnetică nucleară a nucleilor de carbon 13 – ^{13}C-RMN, spectroscopie de masă de înaltă rezoluție – HRMS). Potențialul antioxidant al compușilor sintetizați a fost evaluat prin teste *in vitro*: acțiunea antiradicalică asupra DPPH (2,2-difenil-1-picrilhidrazil**)** și ABTS (acid 2,2′-azinobis-(3-etilbenzothiazolin-6-sulfonic)) și capacitatea totală antioxidantă. Rezultatele obținute au evidențiat faptul că, în general, modificarea chimică a teofilinei a fost asociată cu îmbunătățirea efectelor antioxidante ale compusului părinte. Cel mai activ compus a fost hidrazona **c**, care are 2-OH ca substituent pe nucleul aromatic, activitatea sa antiradicalică față de ABTS fiind comparabilă cu cea a acidului ascorbic. Substituția nucleului aromatic cu 3/4-OH și 4-CN a fost de asemenea asociată cu efecte antioxidante îmbunătățite, derivații corespunzători prezentând apreciabile efecte antiradicalice (DPPH, ABTS) și capacitate totală antioxidantă.

Keywords: theophylline, hydrazones, spectral methods, antioxidant effects

Introduction

In the last period of time, the compounds with hydrazone structure have attracted the interest of many researchers for two reasons. Firstly, based on their facile synthesis these compounds are important intermediary products in the development of new molecules with potential biological activity [13]. Secondly, the literature reports an important number of hydrazones which show interesting biological effects, such as antioxidant [12], antitumor [17], anti-inflammatory [8], anticonvulsive [10], analgesic [6], antimicrobial [7, 14] and antiviral [18].

In this study, starting from theophylline, several hydrazone derivatives have been synthesized. Xanthine derivatives, including theophylline, are known for important biological effects such as broncho-dilatory, anti-inflammatory, antioxidant, hypo-glycemiant and anticancer [9]. It is proved that the reactive oxygen species, which are involved in production of oxidative stress, are associated with the damage of the cells [5]. Based on the role of oxidative stress in many diseases, the synthesized hydrazones derivatives were evaluated for their *in vitro* antioxidant effects.

Materials and Methods

Reagents. Theophylline sodium, ethyl chloroacetate, hydrazine hydrate 64%, aromatic aldehydes (3-

nitro/4-nitro/2-hydroxy/3-hydroxy/4-hydroxy/4-cyan/ 2,6-dimethoxy/2,5-dimethoxy/3,4-dimethoxy/2,3,4-trimethoxy/2,4,5-trimethoxy/3,4,5-trimethoxy/2,4,6-trimethoxy-benzaldehyde), organic solvents (ethanol, methanol, acetone, dichloromethane, diethyl ether, dimethylformamide (DMFA), ethyl acetate, *n*-pentane - pro analysis (p.a.) quality), catalyst (acetic acid), dimethylsulphoxide-d_6 (DMSO-d_6), standard reagents for the antioxidant assays were purchased from Sigma Aldrich Company. All reagents and solvents were used without prior purification.

Synthesis. The synthesis of hydrazones with xanthine structure was performed according to the procedure described in our previous paper [4]. The reactions were monitored using Thin Layer Chromatography (TLC) Silica gel 60 F_{254} plates produced by Merck Company and the spots were visualized using UV light.

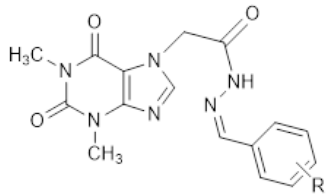
Physico-chemical and spectral characterization. The melting points were measured using a Buchi Melting Point B-540 apparatus and they are un-corrected. The infrared (IR) spectra were recorded on a Thermo-Nicolet AVATAR 320 AEK0200713 FT-IR Spectrometer, at a resolution of 4 cm^{-1} after 6 scans in the 4000-500 cm^{-1} range. The spectra were processed using the Omnic Spectra Software. The ^{1}H-NMR (400 MHz) and ^{13}C-NMR (101 MHz) spectra were recorded with a Bruker Avance Spectrometer 400 MHz, using tetramethylsilane (TMS) as internal standard and DMSO-d_6 as solvents produced by Sigma Aldrich Company. The chemical shifts were shown in δ values (ppm). The mass spectra were registered using a Bruker MaXis Ultra-High Resolution Quadrupole Time-of-Flight Mass Spectrometer.

Biological evaluation. The antioxidant activity was evaluated using *in vitro* assays: the scavenging ability against DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)) radicals and total antioxidant capacity assays, according to the procedure described in the literature [2, 11, 16] with slight modifications. The methods are based on measurements of absorbance of the specific complex, using a GBC Cintra 2010 UV-VIS Spectrophotometer and the values were recorded using Cintral Software. The results are expressed as scavenging ability (%) and efficient concentration 50 (EC_{50}) values. The theophylline (Th) and ascorbic acid (AA) were used as reference and positive control respectively.

Results and Discussion

Physico-chemical and spectral characterization

The hydrazones with xanthine structure (Figure 1) are amorphous powders or fluffy solid with cotton structure and white to beige colour. The compounds exist as a mixture of two tautomers in dynamic equilibrium, with different ratio between isoforms (9/1, 8/2).



(**a-m**)

Figure 1.

General structure of hydrazones with xanthine structure

They are soluble in DMSO, DMFA, dioxane and a mixture of acetone with water (at heating), but insoluble in water, ethanol, ethyl acetate and diethyl ether.

The physical and chemical characteristics of the synthesized compounds, such as formula, yield (η), the ratio between isoforms and melting point (m.p.) are presented in Table I.

Table I

Physico-chemical characteristics of hydrazones with xanthine structure (**a-m**)

No.	R	Formula	η (%)	Isomers ratio	m. p. (ºC)	$[M+H]^+$ calculated	$[M+H]^+$ found
a	3-NO_2	$C_{16}H_{15}N_7O_5$*	77*	8/2	295 - 296*	386.120743	386.120644
b	4-NO_2	$C_{16}H_{15}N_7O_5$*	87*	8/2	305 - 308*	386.120743	386.120751
c	2-OH	$C_{16}H_{16}N_6O_4$	90	9/1	284	357.130579	357.130312
d	3-OH	$C_{16}H_{16}N_6O_4$*	94*	8/2	295 - 296*	357.130579	357.130503
e	4-OH	$C_{16}H_{16}N_6O_4$	79	8/2	283	357.130579	357.130464
f	4-CN	$C_{17}H_{15}N_7O_3$	95	8/2	292	366.130914	366.130957
g	2,6-OCH_3	$C_{18}H_{20}N_6O_5$	91	9/1	294	401.156794	401.156508
h	2,5-OCH_3	$C_{18}H_{20}N_6O_5$	77	8/2	284	401.156794	401.156572
i	3,4-OCH_3	$C_{18}H_{20}N_6O_5$	89	8/2	294	401.156794	401.156690
j	2,3,4-OCH_3	$C_{19}H_{22}N_6O_6$	61	8/2	260 - 262	431.167359	431.167215
k	2,4,6-OCH_3	$C_{19}H_{22}N_6O_6$	94	9/1	275	431.167359	431.167230
l	2,4,5-OCH_3	$C_{19}H_{22}N_6O_6$	88	8/2	284	431.167359	453.149303
m	3,4,5-OCH_3	$C_{19}H_{22}N_6O_6$	59	8/2	274 - 275	431.167359	431.167193

*the data are presented in our previous paper [4]

The structure of the compounds was proved using spectral methods (FT-IR, ^{1}H-NMR, ^{13}C-NMR, HRMS). In the IR spectra the specific azomethine bond (CH=N) appears between 1610 - 1588 cm^{-1}, while the CO-NH and NH groups resonated in the region of 1664 - 1640 cm^{-1} and 3217 - 3066 cm^{-1} respectively.

The proton of the azomethine groups (N=CH) resonates in the ^{1}H-NMR spectra at 7.94 - 8.52 ppm for one form and at 8.02 - 8.53 ppm for the other form. The signal of the amide proton (CO-NH) appears at 11.35 - 12.07 ppm for hydrazone and at 11.45 - 12.07 ppm for the tautomer form. The carbon of the two azomethine groups (N=CH) resonates between 139.85 - 148.07 ppm. The structure proposed for the synthesized compounds is strongly supported by the spectral mass (Table I) coupled with the NMR data.

N-(3-Nytrobenzylidene)-2-(1,3-dimethylxanthin-7-yl)-acethydrazide (**a**): ^{13}C-NMR (101 MHz, DMSO-d$_6$, δ ppm): 168.70/163.78 (C$_q$), 154.91 (C$_q$), 151.47 (C$_q$), 148.73/148.63 (C$_q$), 148.32/148.44 (C$_q$), 144.06/145.47 (CH=N), 142.62/144.2 (CH=N), 136.16/136.36 (C$_q$), 133.47/133.64 (CH$_{Ar}$), 130.89 (CH$_{Ar}$), 124.75/124.84 (CH$_{Ar}$), 121.15/121.73 (CH$_{Ar}$), 107.15/106.87 (C$_q$), 47.86/47.99 (N-CH$_2$-CO), 29.91 (N-CH$_3$), 27.87/27.89 (N-CH$_3$).

N-(4-Nytrobenzylidene)-2-(1,3-dimethylxanthin-7-yl)-acethydrazide (**b**): ^{13}C-NMR (101 MHz, DMSO-d$_6$, δ ppm): 168.83/163.86 (C$_q$), 154.92 (C$_q$), 151.48 (C$_q$), 148.34/148.45 (C$_q$), 148.33 (C$_q$), 144.83/148.41 (C$_q$), 144.07/145.43 (CH=N), 142.49/144.19 (CH=N), 140.56/140.77 (C$_q$), 128.36/128.55 (CH$_{Ar}$), 124.50 (CH$_{Ar}$), 107.14/106.87 (C$_q$), 47.86/48.04 (N-CH$_2$-CO), 29.93 (N-CH$_3$), 27.88 (N-CH$_3$).

N-(2-Hydroxybenzylidene)-2-(1,3-dimethylxanthin-7-yl)acethydrazide (**c**): IR (ATR diamond, cm^{-1}): 3121 (NH), 3057 (OH), 2987 (CH$_{Ar}$), 1640 (CO-NH), 1608 (CH=N), 1123 (O-C); ^{1}H-NMR (400 MHz, DMSO-d$_6$, δ ppm): 11.68/11.98 (s, 1H, CO-NH), 10.05/10.90 (s, 1H, -OH), 8.36/8.44 (s, 1H, CH=N), 8.05/8.08 (s, 1H, CH=N), 7.75 (d, *J* = 6.7 Hz, 1H, Ar-H)/7.55 (d, *J* = 6.8 Hz, 1H, Ar-H), 6.95–6.83/7.32–7.23 (m, 3H, Ar-H), 5.53/5.14 (s, 2H, CH$_2$-CO), 3.46 (s, 3H, N-CH$_3$), 3.20 (s, 3H, N-CH$_3$); ^{13}C-NMR (101 MHz, DMSO-d$_6$, δ ppm): 168.06/163.22 (C$_q$), 156.91/157.70 (C$_q$), 154.93 (C$_q$), 151.49 (C$_q$), 148.31/148.47 (C$_q$), 144.11/147.86 (CH=N), 141.97/144.22 (CH=N), 131.82/132.01 (CH$_{Ar}$), 126.49/129.5 (CH$_{Ar}$), 120.48/119.09 (C$_q$), 119.88/119.83 (CH$_{Ar}$), 116.65/116.80 (CH$_{Ar}$), 107.17/106.88 (C$_q$), 47.80/47.85 (N-CH$_2$-CO), 29.92/29.94 (N-CH$_3$), 27.88/27.92 (N-CH$_3$).

N-(3-Hydroxybenzylidene)-2-(1,3-dimethylxanthin-7-yl)acethydrazide (**d**): ^{13}C-NMR (101 MHz, DMSO-d$_6$, δ ppm): 168.28/163.27 (C$_q$), 158.13 (C$_q$), 154.94 (C$_q$), 151.49 (C$_q$), 148.32/148.43 (C$_q$), 144.97/147.87 (CH=N), 144.14/144.22 (CH=N), 135.54/135.69 (C$_q$), 130.37/130.31 (CH$_{Ar}$), 118.89/119.36 (CH$_{Ar}$), 117.88/118.02 (CH$_{Ar}$), 113.16/113.04 (CH$_{Ar}$), 107.15/106.88 (C$_q$), 47.76/47.94 (N-CH$_2$-CO), 29.92 (N-CH$_3$), 27.88/27.91 (N-CH$_3$).

N-(4-Hydroxybenzylidene)-2-(1,3-dimethylxanthin-7-yl)acethydrazide (**e**): IR (ATR diamond, cm^{-1}): 3217 (NH), 3161 (OH), 2987 (CH$_{Ar}$), 1641 (CO-NH), 1608 (CH=N), 1124 (O-C); ^{1}H-NMR (400 MHz, DMSO-d$_6$, δ ppm): 11.54/11.61 (s, 1H, CO-NH), 9.93 (s, 1H, -OH), 8.05/8.10 (s, 1H, CH=N), 7.94/8.06 (s, 1H, CH=N), 7.55/7.51 (d, *J* = 8.5 Hz, 2H, Ar-H), 6.83 (d, *J* = 8.4 Hz, 2H, Ar-H)/6.83 (d, *J* = 8.4 Hz, 1H, Ar-H) and 6.80 (s, 1H, Ar-H), 5.51/5.09 (s, 2H, CH$_2$-CO), 3.46 (s, 3H, N-CH$_3$), 3.20 (s, 3H, N-CH$_3$); ^{13}C-NMR (101 MHz, DMSO-d$_6$, δ ppm): 167.98/162.87 (C$_q$), 159.86/159.93 (C$_q$), 154.94/154.91 (C$_q$), 151.49 (C$_q$), 148.30/148.43 (C$_q$), 145.02/148.07 (CH=N), 144.12/144.22 (CH=N), 129.12/129.36 (CH$_{Ar}$), 125.32/125.38 (C$_q$), 116.17/116.13 (CH$_{Ar}$), 107.17/106.88 (C$_q$), 47.81/47.90 (N-CH$_2$-CO), 29.92 (N-CH$_3$), 27.88/27.91 (N-CH$_3$).

N-(4-Cyanobenzylidene)-2-(1,3-dimethylxanthin-7-yl)acethydrazide (**f**): IR (ATR diamond, cm^{-1}): 3073 (NH), 2951 (CH$_{Ar}$), 1656 (CO-NH), 1597 (CH=N), 1552 (C-N); ^{1}H-NMR (400 MHz, DMSO-d$_6$, δ ppm): 12.00 (s, 1H, CO-NH), 8.10/8.28 (s, 1H, CH=N), 8.06/8.07 (s, 1H, CH=N), 7.95-7.88 (m, 4H, Ar-H), 5.58/5.14 (s, 2H, CH$_2$-CO), 3.46 (s, 3H, N-CH$_3$), 3.19 (s, 3H, N-CH$_3$); ^{13}C-NMR (101 MHz, DMSO-d$_6$, δ ppm): 168.80/162.97 (C$_q$), 154.92 (C$_q$), 151.49 (C$_q$), 148.34/148.45 (C$_q$), 144.07/144.20 (CH=N), 142.86 (CH=N), 138.77/139.03 (C$_q$), 133.22 (CH$_{Ar}$), 127.99/128.16 (CH$_{Ar}$), 119.10 (C$_q$), 112.38/112.46 (C$_q$), 107.15 (C$_q$), 47.87/48.03 (N-CH$_2$-CO), 29.94 (N-CH$_3$), 27.89 (N-CH$_3$).

N-(2,6-Dimethoxybenzylidene)-2-(1,3-dimethylxanthin-7-yl)acethydrazide (**g**): IR (ATR diamond, cm^{-1}): 3085 (NH), 2951 (CH$_{Ar}$), 1652 (CO-NH), 1600 (CH=N), 1088 (O-C); ^{1}H-NMR (400 MHz, DMSO-d$_6$, δ ppm): 11.51/11.62 (s, 1H, CO-NH), 8.27/8.36 (s, 1H, CH=N), 8.06 (s, 1H, CH=N), 7.34 (t, J = 8.4 Hz, 1H, Ar-H), 6.72 (d, J = 8.4 Hz, 2H, Ar-H)/6.71 (t, *J* = 7.9 Hz, 2H, Ar-H), 5.44/5.09 (s, 2H, CH$_2$-CO), 3.83/3.78 (s, 6H, O-CH$_3$), 3.45 (s, 3H, N-CH$_3$), 3.20/3.21 (s, 3H, N-CH$_3$); ^{13}C-NMR (101 MHz, DMSO-d$_6$, δ ppm): 168.15/162.74 (C$_q$), 159.28/159.11 (C$_q$), 154.91 (C$_q$), 151.48 (C$_q$), 148.28/148.41 (C$_q$), 144.23/143.37 (CH=N), 139.85 (CH=N), 131.73/131.79 (CH$_{Ar}$), 110.97/111.10 (C$_q$), 107.15/106.87 (C$_q$), 104.94/104.80 (CH$_{Ar}$), 56.53/56.40 (O-CH$_3$), 47.86 (N-CH$_2$-CO), 29.91 (N-CH$_3$), 27.88/27.92 (N-CH$_3$).

N-(2,5-Dimethoxybenzylidene)-2-(1,3-dimethylxanthin-7-yl)acethydrazide (**h**): IR (ATR diamond, cm^{-1}): 3089 (NH), 2947 (CH$_{Ar}$), 1645 (CO-NH), 1596 (CH=N), 1047 (O-C); ^{1}H-NMR (400 MHz, DMSO-d$_6$, δ ppm): 11.72 (s, 1H, CO-NH), 8.35/8.53 (s, 1H, CH=N), 8.05/8.07 (s, 1H, CH=N), 7.38 (d, *J* = 2.8 Hz, 1H,

Ar-H)/7.27 (d, *J* = 2.9 Hz, 1H, Ar-H), 7.04/7.07 (s, 1H, Ar-H), 7.02 (d, *J* = 2.9 Hz, 1H, Ar-H)/7.00 (d, *J* = 3.0 Hz, 1H, Ar-H), 5.55/5.09 (s, 2H, CH_2-CO), 3.81 (s, 3H, O-CH_3), 3.75/3.72 (s, 3H, O-CH_3), 3.45 (s, 3H, N-CH_3), 3.19 (s, 3H, N-CH_3); ^{13}C-NMR (101 MHz, DMSO-d_6, δ ppm): 168.31/163.15 (C_q), 154.90 (C_q), 153.72/153.68 (C_q), 152.65/152.73 (C_q), 151.48 (C_q), 148.30/148.43 (C_q), 144.06/144.19 (CH=N), 140.16/143.22 (CH=N), 122.89 (C_q), 117.75/118.26 (CH_{Ar}), 113.80/113.89 (CH_{Ar}), 110.03/109.63 (CH_{Ar}), 107.18/106.89 (C_q), 56.68/56.74 (O-CH_3), 55.96/55.90 (O-CH_3), 47.89/47.94 (N-$\underline{C}H_2$-CO), 29.92 (N-CH_3), 27.88/27.90 (N-CH_3).

N-(3,4-Dimethoxybenzylidene)-2-(1,3-dimethylxanthin-7-yl)acethydrazide (**i**): IR (ATR diamond, cm^{-1}): 3089 (NH), 2961 (CH_{Ar}), 1661 (CO-NH), 1602 (CH=N), 1137 (O-C); ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 11.65 (s, 1H, CO-NH), 8.06/8.13 (s, 1H, CH=N), 7.97/8.06 (s, 1H, CH=N), 7.35/7.28 (s, 1H, Ar-H), 7.21 (d, J = 6.9 Hz, 1H, Ar-H), 7.02 (d, J = 8.3 Hz, 1H, Ar-H), 5.54/5.10 (s, 2H, CH_2-CO), 3.81 (d, J = 4.4 Hz, 6H, O-CH_3)/3.81 (d, J = 4.4 Hz, 3H, O-CH_3) and 3.79 (s, 3H, O-CH_3), 3.46 (s, 3H, N-CH_3), 3.20 (s, 3H, N-CH_3); ^{13}C-NMR (101 MHz, DMSO-d_6, δ ppm): 168.15/163.03 (C_q), 154.91 (C_q), 151.49 (C_q), 151.16/ 151.27 (C_q), 149.52/149.47 (C_q), 148.32/148.44 (C_q), 144.82/147.95 (CH=N), 144.09/144.20 (CH=N), 127.04/ 127.12 (C_q), 121.86/122.25 (CH_{Ar}), 111.99/111.95 (CH_{Ar}), 108.99/108.94 (CH_{Ar}), 107.20/106.90 (C_q), 56.04 (O-CH_3), 55.96/55.91 (O-CH_3), 47.90 (N-$\underline{C}H_2$-CO), 29.93 (N-CH_3), 27.88/27.91 (N-CH_3).

N-(2,3,4-Trimethoxybenzylidene)-2-(1,3-dimethyl-xanthin-7-yl)acethydrazide (**j**): IR (ATR diamond, cm^{-1}): 3107 (NH), 2946 (CH_{Ar}), 1659 (CO-NH), 1592 (CH=N), 1094 (C-O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 11.65 (s, 1H, CO-NH), 8.23/ 8.38 (s, 1H, CH=N), 8.05 (s, 1H, CH=N), 7.59/7.52 (d, *J* = 8.7 Hz, 1H, Ar-H), 6.91 (d, *J* = 8.5 Hz, 1H, Ar-H), 5.51/5.09 (s, 2H, CH_2-CO), 3.84 (s, 6H, O-CH_3), 3.78 (s, 3H, O-CH_3), 3.45 (s, 3H, N-CH_3), 3.19 (s, 3H, N-CH_3); ^{13}C-NMR (101 MHz, DMSO-d_6, δ ppm): 168.04/162.96 (C_q), 155.61/155.72 (C_q), 154.90/155.00 (C_q), 153.00 (C_q), 151.47 (C_q), 148.29/ 148.42 (C_q), 144.10/144.21 (CH=N), 142.05/141.96 (C_q), 140.63/143.31 (CH=N), 120.95 (CH_{Ar}), 120.46/ 120.39 (C_q), 109.17/109.16 (CH_{Ar}), 107.16/106.87 (C_q), 62.22 (O-CH_3), 60.95 (O-CH_3), 56.49/56.45 (O-CH_3), 47.82/47.90 (N-$\underline{C}H_2$-CO), 29.90 (N-CH_3), 27.87 (N-CH_3).

N-(2,4,6-Trimethoxybenzylidene)-2-(1,3-dimethyl-xanthin-7-yl)acethydrazide (**k**): IR (ATR diamond, cm^{-1}): 3116 (NH), 2942 (CH_{Ar}), 1663 (CO-NH), 1605 (CH=N), 1062 (C-O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 11.35/11.45 (s, 1H, CO-NH), 8.21/8.33 (s, 1H, CH=N), 8.06 (s, 1H, CH=N), 6.29/6.27 (s, 2H, Ar-H), 5.42/ 5.06 (s, 2H, CH_2-CO), 3.83 (s, 9H, O-CH_3), 3.45 (s, 3H, N-CH_3), 3.20 (s, 3H, N-CH_3); ^{13}C-NMR (101 MHz, DMSO-d_6, δ ppm): 167.88 (C_q), 162.69/ 162.86 (C_q), 160.44/ 160.33 (C_q), 154.93 (C_q), 151.51 (C_q), 148.29/148.42 (C_q), 144.25/144.28 (CH=N), 139.97 (CH=N), 107.18 (C_q), 104.15 (C_q), 91.66/91.57 (CH_{Ar}), 56.53/56.41 (O-CH_3), 55.90 (O-CH_3), 47.94/47.87 (N-$\underline{C}H_2$-CO), 29.93/ 29.92 (N-CH_3), 27.88/27.89 (N-CH_3).

N-(2,4,5-Trimethoxybenzylidene)-2-(1,3-dimethyl-xanthin-7-yl)acethydrazide (**l**): IR (ATR diamond, cm^{-1}): 3120 (NH), 2942 (CH_{Ar}), 1664 (CO-NH), 1602 (CH=N), 1051 (C-O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 11.54 (s, 1H, CO-NH), 8.29/8.46 (s, 1H, CH=N), 8.04/8.05 (s, 1H, CH=N), 7.36/7.26 (s, 1H, Ar-H), 6.73 (s, 1H, Ar-H), 5.52/5.06 (s, 2H, CH_2-CO), 3.85 (s, 6H, O-CH_3), 3.75/3.71 (s, 3H, O-CH_3), 3.44 (s, 3H, N-CH_3), 3.18 (s, 3H, N-CH_3); ^{13}C-NMR (101 MHz, DMSO-d_6, δ ppm): 167.98/ 162.78 (C_q), 154.87 (C_q), 153.74/153.82 (C_q), 152.52/ 152.63 (C_q), 151.46/151.46 (C_q), 148.27/148.41 (C_q), 144.03/144.16 (CH=N), 143.66 (C_q), 140.40/143.47 (CH=N), 113.55/113.50 (C_q), 108.36/108.08 (CH_{Ar}), 107.18/106.88 (C_q), 98.24/98.28 (CH_{Ar}), 56.94/57.00 (O-CH_3), 56.48 (O-CH_3), 56.24/56.31 (O-CH_3), 47.91 (N-$\underline{C}H_2$-CO), 29.90 (N-CH_3), 27.86/27.88 (N-CH_3).

N-(3,4,5-Trimethoxybenzylidene)-2-(1,3-dimethyl-xanthin-7-yl)acethydrazide (**m**): IR (ATR diamond, cm^{-1}): 3066 (NH), 2958 (CH_{Ar}), 1659 (CO-NH), 1608 (CH=N), 1123 (C-O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 11.80 (s, 1H, CO-NH), 8.07/8.14 (s, 1H, CH=N), 7.96/8.07 (s, 1H, CH=N), 7.05/7.01 (s, 2H, Ar-H), 5.55/5.12 (s,2H, CH_2-CO), 3.83 (d, *J*=7.1 Hz, 6H, O-CH_3) and 3.70 (s, 3H, O-CH_3)/ 3.82 (d, *J*=7.1 Hz, 9H, O-CH_3), 3.46 (s, 3H, N-CH_3), 3.20 (s, 3H, N-CH_3); ^{13}C-NMR (101 MHz, DMSO-d_6, δ ppm): 168.37/163.07 (C_q), 154.91 (C_q), 153.65/153.61 (C_q), 151.48 (C_q), 148.33/148.44 (C_q), 144.60/144.59 (CH=N), 144.08/144.20 (CH=N), 139.67 (C_q), 129.81 (C_q), 107.19 (C_q), 104.69/104.86 (CH_{Ar}), 60.58 (O-CH_3), 56.43 (O-CH_3), 47.94 (N-$\underline{C}H_2$-CO), 29.93 (N-CH_3), 27.88 (N-CH_3).

Biological evaluation

DPPH Radical Scavenging Assay. The violet DPPH radical, stable in methanol solution, is transformed, after its reduction in the presence of an antioxidant, to a pale yellow product, whose absorbance is measured at 517 nm [10, 15]. The results expressed as scavenging ability (%) at different concentrations (0.4. mg/mL, 0.8 mg/mL, 1.2 mg/mL, 1.6 mg/mL, 2 mg/mL) are presented in Figure 2.

At 2 mg/mL the most active compounds were **b** (R = 4-NO_2), **l** (R = 2,4,5-OCH_3) and **m** (R = 3,4,5-OCH_3), which are about 3-4 times more active than theophylline (Th) (Table II). The results support the favourable influence of *nitro* and *methoxy* on the scavenging ability. Compared with ascorbic acid (AA), the tested compounds were less active.

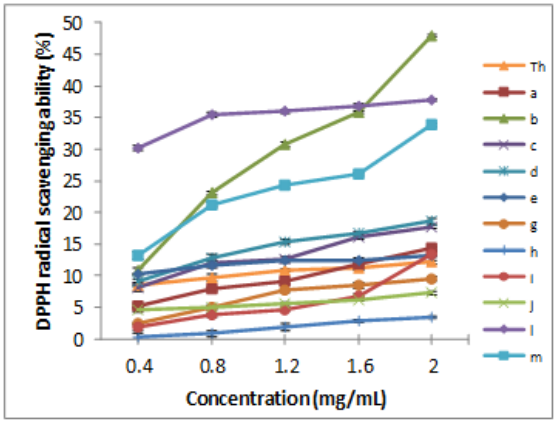


Figure 2.
The DPPH scavenging ability (%) of hydrazones with xanthine structure (**a-m**)

Table II
The DPPH scavenging ability (%) of the tested hydrazones with xanthine structure (**a-m**), reference and control substances

Compound	Scavenging ability (%)
Th	12.14 ± 0.20
a	14.30 ± 0.33
b	47.99 ± 0.22
c	17.78 ± 0.33
d	18.63 ± 0.46
e	13.19 ± 0.27
g	9.47 ± 0.37
h	3.43 ± 0.27
i	13.40 ± 0.34
j	7.28 ± 0.26
l	37.78 ± 0.26
m	33.82 ± 0.26
AA[1]	81.62 ± 0.21

Th = theophylline; AA = ascorbic acid; [1]0.04 mg/mL; Data are mean ± SD (n = 3, p < 0.05)

ABTS radical scavenging ability. The oxidation of ABTS with potassium persulphate generates the $ABTS^{\bullet+}$ radical, a blue chromophore, which is reduced in the presence of hydrogen donating agents. The discoloration of the blue colour was measured at 734 nm [3, 12]. The compounds were tested at different concentrations (0.25 mg/mL, 0.5 mg/mL, 0.75 mg/mL, 1 mg/mL) and the results are presented in Table III. The EC_{50} values of the most active compounds (**c, d, e, l, h**) are indicated in Table IV. The compound **c** (R = 2-OH) showed the best scavenging activity, its EC_{50} value (0.0095 ± 0.0001 mg/mL) being comparable with ascorbic acid (AA) (0.0028 ± 0.0001 mg/mL). A good activity was showed also by compounds **d** (3-OH) and **e** (4-OH). These results support the favourable influence of the hydroxy group on ABTS scavenging ability of the tested compounds.

Table III
The ABTS scavenging ability (%) of hydrazones with xanthine structure (**a-m**)

Compound	Scavenging ability (%)
Th[1]	25.97 ± 0.27
a[1]	17.12 ± 0.11
b[1]	23.01 ± 0.33
c[2]	51.26 ± 0.21
d[3]	61.63 ± 0.34
e[3]	54.31 ± 0.29
f[1]	7.23 ± 0.39
g[1]	44.85 ± 0.15
h[1]	58.35 ± 0.32
i[1]	28.95 ± 0.17
j[1]	18.46 ± 0.35
l[4]	58.20 ± 0.19
AA[5]	78.42 ± 0.40

Th = theophylline; AA = Ascorbic acid; [1]1 mg/mL; [2]0.01 mg/mL; [3]0.1 mg/mL; [4]0.75 mg/mL; [5]0.004 mg/mL; Data are mean ± SD (n = 3, p < 0.05)

Table IV
The ABTS scavenging ability (EC_{50} mg/mL) of the most active compounds

Compound	EC_{50} mg/mL
c	0.0095 ± 0.0001
d	0.0276 ± 0.0006
e	0.0685 ± 0.0038
l	0.4182 ± 0.0100
h	0.7631 ± 0.0065
AA	0.0028 ± 0.0001

Total antioxidant capacity assay. This assay is based on the reduction of Mo(VI) to Mo(V) and the quantitative measurement of its reduced form at 695 nm [1]. The absorbance of tested compounds at different concentrations (0.0291 mg/mL, 0.0582 mg/mL, 0.0872 mg/mL, 0.1163 mg/mL, 0.1745 mg/mL) are represented in Figure 3.

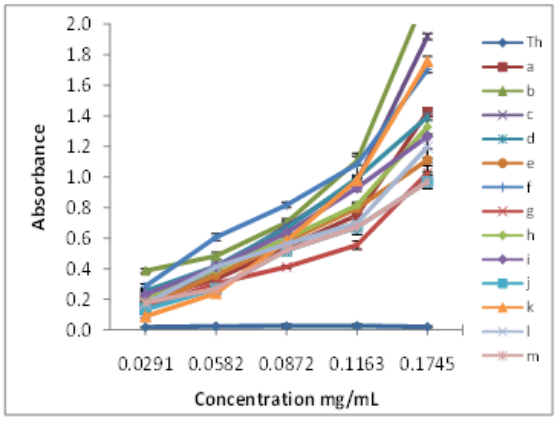


Figure 3.
The absorbance of hydrazones with xanthine structure (**a-m**) and theophylline (Th)

The results expressed as EC_{50} values (mg/mL) are indicated in Table V. The most active compound was **f** (R = 4-CN), for which the EC_{50} was 0.0485 ± 0.0018. A very good activity showed also derivatives **b** (R = 4-NO_2), **d** (R = 3-OH) and **c** (2-OH), which means that *nitro* and *hydroxy* groups have a good influence on total antioxidant capacity. All tested compounds were more active than theophylline (Th), but less active than ascorbic acid (AA), used as positive control.

Table V

The total antioxidant capacity (EC_{50} mg/mL) of hydrazones (**a-m**) and reference and control substance

No	EC_{50} (mg/mL)
Th	*nd*
a	0.0814 ± 0.0010
b	0.0603 ± 0.0033
c	0.0690 ± 0.0014
d	0.0678 ± 0.0011
e	0.0767 ± 0.0043
f	0.0485 ± 0.0018
g	0.1056 ± 0.0040
h	0.0735 ± 0.0019
i	0.0692 ± 0.0053
j	0.0848 ± 0.0030
k	0.0803 ± 0.0015
l	0.0752 ± 0.0011
m	0.0827 ± 0.0042
AA	0.0148 ± 0.0001

Th = theophylline; AA = Ascorbic acid; Data are mean ± SD (n = 3, $p < 0.05$)

Conclusions

In this research, several hydrazones with xanthine structure have been synthesized. The compounds were characterized in terms of physical properties (yield, melting point and solubility in different organic solvents) and their structure was proved through spectral methods (IR, ^{1}H-NMR, ^{13}C-NMR, HRMS). The antioxidant potential was evaluated using *in vitro* assays: DPPH and ABTS radical scavenging ability and total antioxidant capacity assay. The results support that the most part of the tested compounds are more active than theophylline, used as parent compound. Referring to the influence of aromatic substitution on radical scavenging ability it is noted that hydroxy is related with increasing of the ABTS scavenging ability while the *nitro*, especially in para position, is closely related with DPPH scavenging ability. Both substituents seem to be in a good agreement with total antioxidant capacity. An intense antioxidant capacity was showed also by the compound **f** which contains *cyan* as substituent on the aromatic ring.

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