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Antioxidant activities of thiosemicarbazones from substituted benzaldehydes and *N*-(tetra-*O*-acetyl-β-D-galactopyranosyl)thiosemicarbazide

Dinh Thanh Nguyen [a,*], The Hoai Le [a], Thi Thu Trang Bui [b]

[a] *Faculty of Chemistry, VNU University of Science, Ha Noi 10000, Viet Nam*
[b] *Hanoi University of Agriculture, Ha Noi, Viet Nam*

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ABSTRACT

Reaction of *N*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)thiosemicarbazide and different substituted benzaldehydes gave some new substituted benzaldehyde *N*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)thiosemicarbazones. The reaction was performed using conventional and microwave-assisted heating methods. The structures of thiosemicarbazones were confirmed by spectroscopic (IR, ^{1}H NMR, ^{13}C NMR and ESI-MS) method. The antioxidant activity of these thiosemicarbazones was evaluated *in vitro* and *in vivo*, and it's shown that some of these compounds had significant antioxidant activity. Amongst the compounds screened for antioxidant activity, thiosemicarbazones **4a**, **4b** and **4c** showed good antioxidant activity on DPPH. The compounds **4g**, **4i**, **4l** caused significant elevation of SOD activity and **4e**, **4g**, **4i**, **4l** had higher catalase activity, and only compounds **4c** and **4f** expressed the GSH-Px activity.

1. Introduction

Monosaccharides and disaccharides, which contain sulfur, such as isothiocyanates, thioureas, thiosemicarbazides, are versatile precursors in organic synthesis in carbohydrate chemistry [1,2]. On the another hand, thiosemicarbazones, which have NH–C(=S)NH=C bond, are a class of compounds that have been evaluated over the last 50 years as antivirals and as anticancer therapeutics [3]. The chemistry of thiosemicarbazide derivatives of saccharides is interested because these derivatives could be as versatile intermediates for preparing various (e.g., heterocyclic) derivatives as well [4,5] as be used for making complexes formation of metallic ions [6–15]. Thiosemicarbazones exhibit various biological activities such as antituberculosis [16,17], antimicrobial [11,18–20], anti-inflammatory [21], anticonvulsant [11,22], antihypertensive [23], local anesthetic [24], anticancer [12,27], hypoglycemic [28], and cytotoxic activities [11], also antioxidant agents [13,29].

A number of glycosyl thiosemicarbazide and thiosemicarbazones derivatives showed significant *in vivo* anti-microorganisms and *in vitro* antioxidant activity [4,22,25,26], which could be used as leads for the development of effective anti-atherosclerotic agents [29]. On the other hand these molecules can also serve as phosphane-free multidentate ligands for transition-metal catalysis, and they are efficient ligands for palladium-catalyzed coupling reactions in air [15]. In the past some papers have been published for the synthesis of aldehyde/ketone *N*-(per-*O*-acetylated glycopyranosyl)thiosemicarbazones [4,5,20,29–32]. The main synthetic step for the synthesis of these molecules is being the reaction of *N*-(per-*O*-acetylglycosyl)thiosemicarbazides with the corresponding carbonyl compounds. The synthesis of thiosemicarbazones of aromatic carbonyl compounds containing monosaccharide and disaccharide (such as glucose, galactose, lactose and maltose) is the main researches in our lab. Continuing our studied on the synthesis and the reactivity of per-*O*-acetyl-D-glycopyranosyl isothiocyanate and *N*-(per-*O*-acetyl-D-glycopyranosyl)thiosemicarbazides [31,32], we have reported herein a systematic study for the synthesis and spectral characterization of a series of substituted benzaldehyde *N*-(tetra-*O*-acetyl-β-D-galactopyranosyl)thiosemicarbazones using microwave-assisted method [33].

2. Results and discussion

2.1. Chemistry

The transformation reaction of tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate into corresponding thiosemicarbazide could

* Corresponding author. Tel.: +84 04 3826 1853; fax: +84 04 3824 1140.
E-mail address: nguyendinhthanh@hus.edu.vn (D.T. Nguyen).

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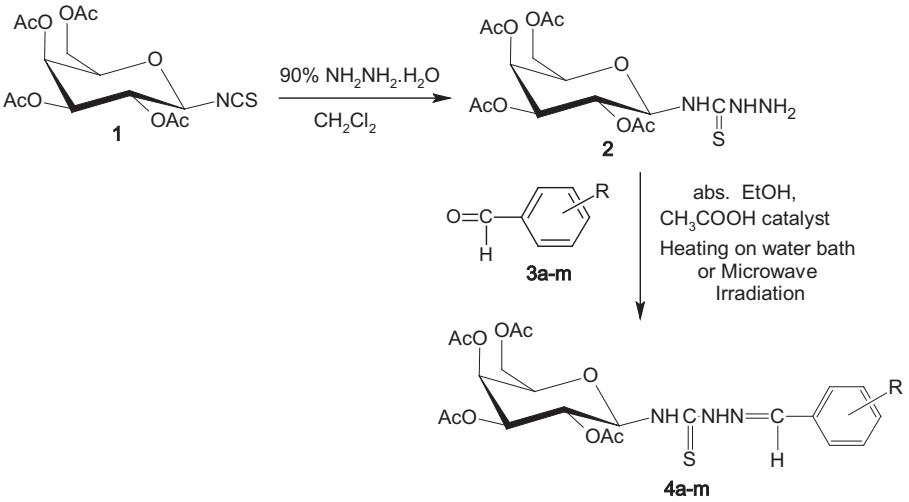
be carried out in different solvents, usually aprotic ones, such as dioxane [34], dichloromethane [29], but a protic one could be used, such as absolute ethanol, so the reaction must be performed at low temperature (<10 °C) to prevent the decomposition of isothiocyanate derivative [35]. We realized that the use of dichloromethane as solvent in this reaction is of great advantage to work out the reaction, due to low boiling point of that solvent. For improvement in this reaction, we have used an 85% solution of hydrazine solution instead of 100% hydrazine hydrate [29]. In this work, *N*-(tetra-*O*-acetyl-β-D-galactopyranosyl)thiosemicarbazide was synthesized from corresponding isothiocyanate derivative by reaction with hydrazine hydrate (Scheme 1) by similar method. After reaction, the solvent was removed under reduced pressure to obtain a syrup residue, sometimes a solid one could be gotten. The trituration of residue with 96% ethanol to give product *N*-(tetra-*O*-acetyl-β-D-galactopyranosyl)thiosemicarbazide due to its low solubility in this solvent.

Condensation reaction of *N*-(tetra-*O*-acetyl-β-D-galactopyranosyl)thiosemicarbazide **2** with a number of substituted benzaldehydes **3a**–**m** lead to form a series of benzaldehyde *N*-(tetra-*O*-acetyl-β-D-galactopyranosyl)thiosemicarbazones **4a**–**m** (Scheme 1). The reaction was performed by using microwave-assisted heating and conventional heating methods. The microwave-assisted synthetic pathway was carried out using minimum amount of solvent (ethanol) and deceased reaction time comparing conventional heating pathway (2–3 mL volume versus 20 mL, and 2–7 min versus 90 min, respectively). Reaction time was from 2 to 7 min depending on substituent's nature: withdrawing substituents need shorter reaction time than donating ones. When reaction was starting to irradiate about 1–3 min, the pasty mixture of reagents in methanol was dissolved and the reaction became homogenous. Finally, the solid product appeared and precipitated out. The products yields of microwave-assisted method were fairly high from 60 to 98%, while ones of conventional heating methods were lower, from 32 to 64%. In some cases with benzaldehydes having 4-Cl, 4-NO_2 and 4-Br groups the yields attained 98%. These compounds can dissolved in ethanol toluene, chloroform, *N*,*N*-dimethylformamide, and have high melting points (Table 1). The synthesized products were characterized by IR, ^{1}H NMR and ^{13}C NMR spectral data.

The IR spectra of compounds **4a**–**m** showed characteristic absorptions in the range of 3354–3313 cm^{-1} (N–H bond), 1752–1744, 1261–1216 and 1055–1045 cm^{-1} (ester), 1370–1378 cm^{-1} (C=S), and 1625–1587 cm^{-1} (CH=N bond). The anomeric proton H-1 is represented as a triplet at δ = 5.90–5.95 ppm due to the coupling with both H-4″ and H-2″ protons in the ^{1}H NMR spectra of **4a**–**m**. The coupling constant values, $J_{H\text{-}1,\ H\text{-}2}$ = 9.0–9.5 Hz, for the pyranose ring agreed with *trans*-axial H–H disposition and confirmed the β-anomeric configuration of compounds **4a**–**m**. Signals of NH protons of the thiourea component in compounds **4a**–**m** appeared at δ = 12.17–11.71 ppm (in singlet) for H-2 and δ = 9.00–8.43 ppm (in doublet, $J_{NH,\ H\text{-}1}$ = 9.5–8.5 Hz) for H-4. Proton of azomethine bond had chemical shift at δ = 8.22–7.98 ppm in singlet. Other protons in pyranose ring had signals in region of 5.93–4.03 ppm. Protons in benzene ring appeared at δ = 8.27–6.73 ppm. The ^{13}C NMR spectra showed the thiocarbonyl carbon atom with chemical shift at δ = 178.84–177.25 ppm. Carbon atom of azomethine bond showed chemical shift at δ = 159.70–142.56 ppm. Carbon atoms of benzene and pyranose rings had signals at δ = 159.58–111.11 and δ = 81.94–61.10 ppm, respectively. Acetate ester in sugar component had signals at δ = 20.51–20.26 and δ = 170.53–169.24 ppm for carbon atoms in methyl and

Table 1
Synthetic conditions for compounds **4a**–**m**.

Compd	R	Microwave-assisted method			Conventional method		
		Reaction time, min	Ethanol solvent, mL	Yield, %	Reaction time, min	Ethanol solvent, mL	Yield, %
4a	4-NMe_2	7	3	74	90	20	64
4b	3-OEt–4-OH	7	3	80			
4c	3-OMe–4-OH	7	3	70			
4d	3-OH–4-OMe	7	3	75			
4e	3-OMe	5	2	85			
4f	4-OH	5	3	75			
4g	4-^{i}Pr	5	2	75			
4h	4-Me	5	2	60			
4i	4-Br	5	2	98			
4j	4-Cl	5	2	98	90	20	32
4k	4-F	5	2	73			
4l	3-NO_2	5	3	70	90	20	60
4m	4-NO_2	5	3	97	90	20	48



Scheme 1. The synthesis route for preparation of the title compounds **4a**–**m**.

carbonyl groups, respectively. Protons in methyl group of acetate ester had chemical shifts at $\delta = 2.16–1.93$ ppm.

2.2. Study on anti-oxidant activity

The *in vitro* method of the scavenging of the stable DPPH radical is extensively used to evaluate antioxidant activities in less time than other methods [36–39]. DPPH is a stable free radical molecule that can accept an electron or hydrogen radical and thus be converted into a stable, diamagnetic molecule. DPPH has an odd electron and so has a strong absorption band at 518 nm. When this electron becomes paired off, the absorption decreases stoichiometrically with respect to the number of electrons taken up. Such a change in the absorbance produced in this reaction has been widely applied to test the capacity of numerous molecules to act as free radical scavengers. The scavenging effect of the synthesized compounds **4a**–**m** on the DPPH radical was evaluated according to the methods of Shimada et al. [37], Leong and Shui [38] and Braca et al. [39]. Antioxidant activity of synthesized compounds by DPPH method were shown in Table 2 and Fig. 1.

The reaction mechanism of thiosemicarbazones **4a**–**m** with DPPH radical was suggested at Scheme 2. In the DPPH radical scavenging effect assay, when the concentration of thiosemicarbazones **4** was higher than that of DPPH radical, the quantity of these thiosemicarbazones **4a**–**m** was enough to consume the DPPH radical and the stoichiometry of this reaction was 1:1 shown in Scheme 2, Eq. (I). The reaction of the DPPH radical may be based either on a charge transfer with tested compounds perhaps initiated by DPPH radical [Eq. (I)] or on a combination of the DPPH radical with thiosemicarbazone radical formed during the DPPH radical scavenging assay [Eq. (II)]. A reaction of DPPH molecules with each other is not possible due to their steric hindrance [40].

The scavenging activity of compounds **4a**–**m** are perhaps due to the presence of an N–H group in the thiosemicarbazone moiety, which can donate a hydrogen atom to the DPPH radical. After donating a hydrogen atom, compounds **4a**–**m** exist in a radical form, and the radical could delocalize to the benzene ring to produce the stable resonance hybrid shown in Scheme 2. The electron conjugation in the structure stabilizes the radical, preventing it from participating in a destructive biochemical reaction. Amongst the compounds screened for antioxidant activity, **4a**, **4b**, **4c** and **4f** showed good antioxidant activity on DPPH (2,2-diphenyl-1-picrylhydrazyl radical, DPPH•). The compounds with substituents such as 4-NMe_2 (**4a**), 3-OEt-4-OH (**4b**), 3-OMe-4-OH (**4c**) and 4-OH (**4f**) showed very good antioxidant activity on this radical. Remained compounds do not show any antioxidant activity comparing with resveratrol (Table 2, Figs. 2 and 3).

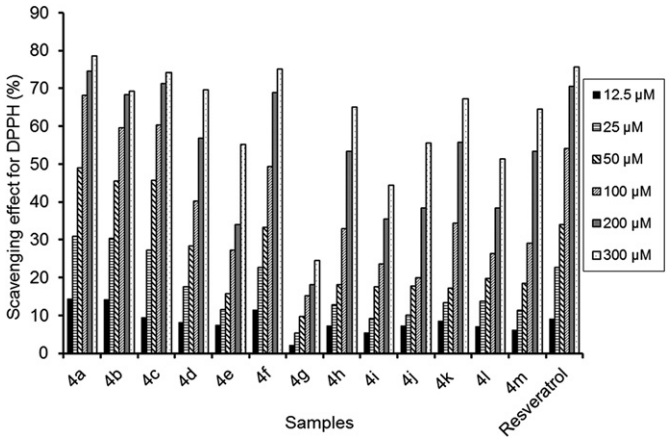


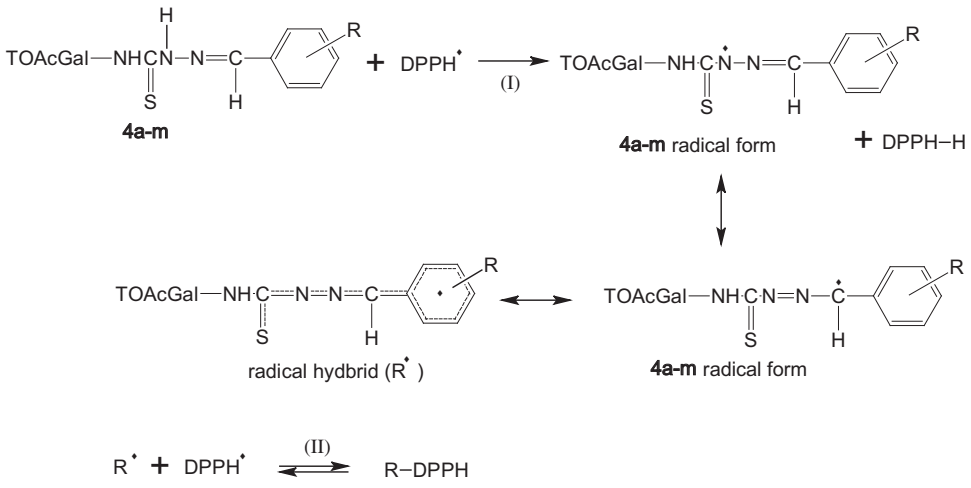
Fig. 1. DPPH radical scavenging capacity (%) of compounds **4a**–**m** at different concentrations (μM). Resveratrol was used as a reference.

When the concentration of the tested compounds was lower than that of DPPH radical, the residual DPPH radical might combine with the resulting thiosemicarbazones radical **4** shown in Scheme 2, Eq. (II), and the stoichiometry of this reaction seemed to be higher than 1:1 in some case. Besides that, thiosemicarbazones with substituents such as 4-NMe_2 (**4a**), 3-OEt-4-OH (**4b**), 3-OMe-4-OH (**4c**) and 4-OH (**4f**) behaved the stronger DPPH radical scavenging activity than remain others. The reason of this phenomena is that the electron resonance effect of substituted benzene ring in radical **4a**, **4b**, **4c** and **4f** making the radical more stable in the presence of electron-donating groups. The result of DPPH radical scavenging activity of all compounds **4a**–**m** was summarized by IC_{50} shown in Table 2.

Compounds **4a**–**m** was tested *in vivo* for their anti-oxidant activities and the results are shown in Table 3. These compounds, when administered i.p., with a dry weight equivalent dosage of 100 mg/kg/day of total extract for seven consecutive days in the CCl_4-intoxicated rats, was shown to cause a significant elevation of free radical scavenging enzyme activities such as superoxide dismutases, catalase, and glutathione peroxidase. Superoxide dismutase (SOD, EC 1.15.1.1) are enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide [41]. Thus, they are an important antioxidant defense in nearly all cells exposed to

Table 2
Antioxidant activity of synthesized compounds by DPPH method.

Compound	Scavenging effect for DPPH (%)						IC_{50} (μM)
	Concentration (μM)						
	12.5	25	50	100	200	300	
4a	14.32 ± 2.23	30.86 ± 2.54	48.94 ± 2.75	68.17 ± 2.66	74.54 ± 2.43	78.47 ± 2.55	56
4b	14.16 ± 3.21	30.24 ± 3.41	45.38 ± 3.73	59.42 ± 3.85	68.34 ± 3.23	69.16 ± 3.34	71
4c	9.45 ± 2.15	27.11 ± 2.59	45.64 ± 2.34	60.30 ± 2.74	71.23 ± 2.65	74.05 ± 2.62	75
4d	8.16 ± 1.18	17.43 ± 1.34	28.21 ± 1.23	40.09 ± 1.51	56.80 ± 1.45	69.61 ± 1.51	182
4e	7.34 ± 1.34	11.46 ± 1.55	15.63 ± 1.23	27.17 ± 1.34	34.02 ± 1.45	55.07 ± 1.48	276
4f	11.45 ± 3.54	22.61 ± 3.78	33.27 ± 3.65	49.18 ± 3.62	68.74 ± 3.67	75.08 ± 3.71	108
4g	2.17 ± 1.32	5.32 ± 1.53	9.65 ± 1.48	15.09 ± 1.56	18.13 ± 1.24	24.48 ± 1.43	>300
4h	7.21 ± 1.31	12.76 ± 1.56	18.06 ± 1.82	32.84 ± 1.78	53.27 ± 1.67	65.03 ± 1.63	206
4i	5.38 ± 1.43	9.04 ± 1.85	17.46 ± 1.51	23.51 ± 1.60	35.42 ± 1.49	44.31 ± 1.42	>300
4j	7.15 ± 1.55	10.09 ± 1.78	17.61 ± 1.73	19.82 ± 1.83	38.37 ± 1.78	55.42 ± 1.72	270
4k	8.51 ± 1.42	13.32 ± 1.67	17.08 ± 1.55	34.34 ± 1.63	55.63 ± 1.59	67.19 ± 1.54	197
4l	7.05 ± 1.50	13.74 ± 1.58	19.63 ± 1.62	26.29 ± 1.57	38.31 ± 1.49	51.24 ± 1.41	283
4m	6.11 ± 1.93	11.32 ± 1.75	18.47 ± 1.87	29.08 ± 1.89	53.30 ± 1.80	64.46 ± 1.81	210
Resveratrol	9.13 ± 1.59	22.56 ± 1.95	33.84 ± 1.79	54.03 ± 1.65	70.44 ± 1.55	75.62 ± 1.49	94



Scheme 2. Reaction of compounds **4a**–**m** with DPPH radical.

oxygen. Catalase is a common enzyme found in nearly all living organisms exposed to oxygen and catalyzes the decomposition of hydrogen peroxide to water and oxygen. Catalase has one of the highest turnover numbers of all enzymes; one catalase molecule can convert millions of molecules of hydrogen peroxide to water and oxygen each second [42]. Antioxidative enzyme glutathione peroxidase (GSH-Px, EC 1.11.1.9) is the general name of an enzyme family with peroxidase activity whose main biological role is to protect the organism from oxidative damage [43]. The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water. As shown in Table 3 and Fig. 4, some of the compounds **4a**–**m** caused significant elevation of SOD activity. Compounds **4g**, **4i**, **4l** caused significant elevation of SOD activity and **4e**, **4g**, **4i**, **4l** had higher catalase activity. But as showed in Table 3, the SOD activity of **4a**, **4b** and **4c** treated groups showed the lower activity. It can be explained that the compounds of **4a**, **4b** and **4c** could protect the CCl_4 in toxicated rats from oxidant injury but not cause significant elevation of SOD activity. The GSH-Px activity of these compounds had some little picture: almost compounds expressed negligible GSH-Px activity, except compound **4c** (R = 3-OMe–4-OH) and **4f** (4-OH).

3. Conclusion

In conclusion, a series of substituted benzaldehyde *N*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)thiosemicarbazones have been synthesized from *N*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)thiosemicarbazide and substituted benzaldehydes using conventional heating and microwave-assisted heating method. The antioxidant activity of these thiosemicarbazones was evaluated, *in vitro* and *in vivo*, and it's shown that some of these compounds had significant antioxidant activity.

4. Experimental section

All solvents, chemicals, and reagents were obtained commercially and used without purification. Melting points were determined by open capillary method on STUART SMP3 instrument (BIBBY STERILIN, UK) and are uncorrected. IR spectra (KBr disc) were recorded on an Impact 410 FT-IR Spectrometer (Nicolet, USA). ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance Spectrometer AV500 (Bruker, Germany) at 500.13 MHz and 125.77 MHz, respectively, using DMSO-d_6 as solvent and TMS as an internal standard. Chemical shifts, δ, are given in parts per million (ppm), and spin multiplicities are given as s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet) or m (multiplet). Coupling constants, *J*, are expressed in hertz (Hz). ESI-MS spectra were recorded on mass spectrometer LC-MS LTQ Orbitrap XL (Thermo-Scientific, USA) in methanol, using ESI method. The entire microwave heating experiments were conducted under reaction conditions of power and temperature in reflux-heating conditions. Thin-layer chromatography was performed on silica gel pates $60F_{254}$ No. 5715 (Merck, Germany) with EtOAc and light petroleum (bp 60–90 °C) or toluene, and spots were visualized with UV light or iodine vapor. 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl isothiocyanate **1** was prepared by the reaction of tetra-O-acetylated-β-D-galactopyranosyl bromide, which was prepared from D-galactose,

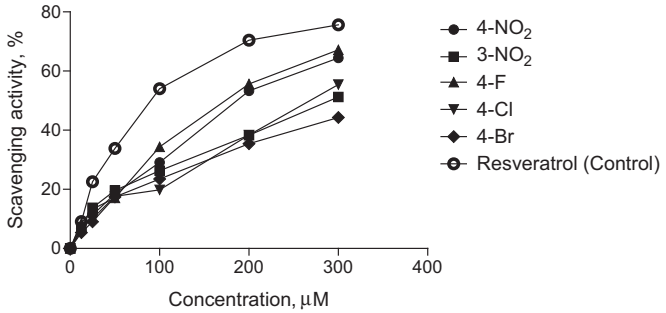


Fig. 2. Scavenging activity of compound **4a**–**e** on DPPH radical.

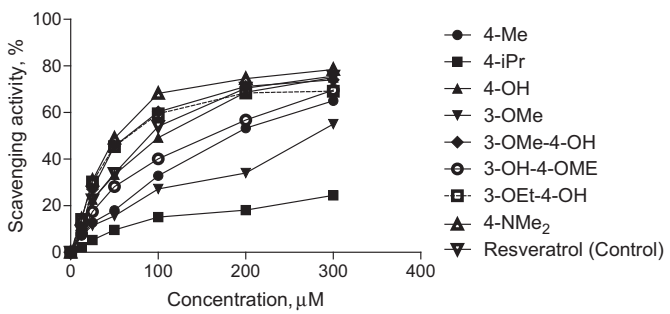


Fig. 3. Scavenging activity of compound **4f**–**m** on DPPH radical.

Table 3
Effect of compounds **4a**–**m** on the liver cytosolic sod, the liver cytosolic GSH-Px, the liver cytosolic catalase activities and the hepatic MDA production.

Compound	SOD (unit/mg protein)	GHS-Px (unit/mg protein)	Catalase (unit/mg protein)
4a	5.81 ± 0.53	0.71 ± 0.02	295.32 ± 10.32
4b	6.45 ± 0.47	0.69 ± 0.02	283.53 ± 12.43
4c	6.57 ± 0.44	0.37 ± 0.04	289.56 ± 13.34
4d	8.76 ± 0.63	0.59 ± 0.03	351.61 ± 11.71
4e	8.89 ± 0.29	0.71 ± 0.01	362.23 ± 11.47
4f	8.24 ± 0.60	0.51 ± 0.02	331.56 ± 10.53
4g	9.92 ± 0.69	1.01 ± 0.01	390.73 ± 12.62
4h	8.82 ± 0.39	0.72 ± 0.02	354.13 ± 11.43
4i	9.95 ± 0.72	0.98 ± 0.01	389.25 ± 12.12
4j	8.91 ± 0.69	0.70 ± 0.01	358.47 ± 12.33
4k	8.60 ± 0.51	0.69 ± 0.01	350.63 ± 12.13
4l	9.01 ± 0.53	0.73 ± 0.01	360.61 ± 11.73
4m	8.79 ± 0.52	0.71 ± 0.02	352.45 ± 12.25
Resveratrol	7.49 ± 0.45	0.35 ± 0.02	285.32 ± 10.26
Control	5.42 ± 0.29	0.27 ± 0.01	218.25 ± 11.43

using the Lemieux's procedure for D-glucose [44], with lead thiocyanate in dried toluene [20].

4.1. Synthesis of N-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl) thiosemicarbazide (**2**)

To a solution of 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl isothiocyanate (10 mmol) in 70 mL of dichloromethane a solution of 85% hydrazine hydrate (10 mmol, 1.2 mL) in 30 mL of dichloromethane was added dropwise with stirring in 30 min at temperature below 20 °C. The temperature of solution was maintained between 15 and 20 °C. The mixture was continued stirring at room temperature for 2 h. The solvent then was removed under reduced pressure to get a yellow solid [32]. The crude product was crystallized from ethanol to yield white product. Yield 71%, mp 197–198 °C; ^{1}H NMR (DMSO-d_6): δ 9.32 (s br, 1H), 8.08 (d, 1H, J = 8.0), 5.76 (t, 1H, J = 9.0), 5.35 (dd, 1H, J = 10.25, 3.75), 5.28 (d, 1H, J = 3.5), 5.09 (t, 1H, J = 9.75), 4.63 (s br, 2H), 4.26 (t, 1H, 6.25), 4.00–3.99 (m, 2H), 2.12 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.93 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 182.1, 170.1, 170.0, 169.9, 169.4, 81.2, 71.2, 70.5, 68.4, 67.6, 61.3, 20.6, 20.5, 20.4, 20.4.

4.2. General procedure for synthesis of substituted benzaldehyde N-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)thiosemicarbazones (**4a**–**m**)

4.2.1. Conventional method (for compounds **4a**, **4b**, **4d** and **4m**)

A suspension mixture of *N*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)thiosemicarbazide 1 (4.21 g, 1 mmol) and corresponding substituted benzaldehyde **3a**, **3b**, **3d** or **3m** (1 mmol) and glacial acetic acid (1 mL) in ethanol (20 mL) was refluxed for 90 min. The solvent was removed under reduced pressure and the residue was triturated with water, the precipitate was filtered by suction and recrystallized from 95% ethanol or 70% ethanol to afford the title compounds of corresponding substituted benzaldehyde *N*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)thiosemicarbazones.

4.2.2. Microwave-assisted method (for all compounds)

A suspension mixture of *N*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)thiosemicarbazide 1 (4.21 g, 1 mmol) and corresponding substituted benzaldehyde **3a**–**m** (1 mmol) and glacial acetic acid (0.05 mL) in absolute ethanol (2–5 mL) was irradiated with reflux for 5–7 min in microwave oven. The suspension mixture became clear solution after irradiating in 3–4 min. After reaction the mixture was cooled to room temperature, the colorless crystals were filtered with suction. The crude product was recrystallized from 95% ethanol or 70% ethanol to afford the title compounds of benzaldehyde *N*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)thiosemicarbazones **4a**–**m**. The physical and spectral (IR, ^{1}H NMR, ^{13}C NMR and ESI-MS) data are in good agreement with their structures.

*4.2.2.1. Synthesis of 4-dimethylaminobenzaldehyde N-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)thiosemicarbazone (**4a**).* White solid, mp 217–218 °C (from 96% ethanol); $[\alpha]_D^{25}$ −97 (*c* 2.0, $CHCl_3$); IR (KBr, cm^{-1}): ν 3343 (NH), 1744 (C=O), 1600 (CH=N), 1223, 1055 (C–O–C); ^{1}H NMR (DMSO-d_6) δ (ppm): 8.43 (d, 1H, J = 9.0 Hz, H-4″), 11.71 (s, 1H, H-2″), 7.99 (s, 1H, H imine), 5.85 (t, 1H, J = 9.5 Hz, H-1), 5.26 (t, 1H, J = 10.0 Hz, H-2), 5.40 (dd, J = 10.0, 3.5 Hz, H-3), 5.34 (d, 1H, J = 3.5 Hz, H-4), 4.31 (t, 1H, J = 6.5 Hz, H-5), 4.05 (d, 1H, 6.5 Hz, H-6), 6.73 (d, 1H, J = 9.0 Hz, H-2′), 7.61 (d, 1H, J = 9.0 Hz, H-3′), 7.61 (d, 1H, J = 9.0 Hz, H-5′), 6.73 (d, 1H, J = 9.0 Hz, H-6′), 1.95–2.15 (s, 1H, CH_3CO); ^{13}C NMR (DMSO-d_6) δ (ppm): 177.3 (C=S), 81.5 (C-1), 68.5 (C-2), 70.4 (C-3), 67.5 (C-4), 71.4 (C-5), 61.2 (C-6), 120.8 (C-1′), 111.6 (C-2′), 128.9 (C-3′), 151.7 (C-4′), 128.9 (C-5′), 111.6 (C-6′), 144.8 (C-imine), 20.3–20.5 (CH_3CO), 169.2–170.1 (CH_3CO), 20.4 [4′-N(CH_3)$_2$]; ESI-MS *m*/*z*: 553 (M + H, 100%), 575 (M + Na, 64%). Anal. Calcd for $C_{24}H_{32}N_4O_9S$ (552.60): C, 52.16; H, 5.84; N, 10.14%. Found: C, 52.19; H, 5.88; N, 10.18%.

*4.2.2.2. Synthesis of 3-ethoxy-4-hydroxybenzaldehyde N-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)thiosemicarbazone (**4b**).* White solid, mp 204–205 °C (from 96% ethanol); $[\alpha]_D^{25}$ −103 (*c* 2.1, $CHCl_3$); IR (KBr, cm^{-1}): ν 3345 (NH), 1747 (C=O), 1600 (CH=N), 1223, 1051 (C–O–C); ^{1}H NMR (DMSO-d_6) δ (ppm): 8.49 (d, 1H, J = 9.0 Hz, H-4″), 11.84 (s, 1H, H-2″), 8.01 (s, 1H, H imine), 5.79 (t, 1H, J = 9.5 Hz, H-1), 5.26 (t, 1H, J = 10.0, H-2), 5.42 (d, 1H, d, J = 10, 4.0 Hz, H-3), 5.35 (d, 1H, J = 3.5 Hz, H-4), 4.32 (t, 1H, J = 6.5 Hz, H-5), 4.04 (m, 1H, H-6), 7.43 (d, 1H, J = 1.5 Hz, H-2′), 6.85 (d, 1H, J = 8.0 Hz, H-5′), 7.15 (dd, 1H, J = 8.0, 1.5 Hz, H-6′), 1.97–2.15 (s, 1H, CH_3CO); ^{13}C NMR (DMSO-d_6) δ (ppm): 177.9 (C=S), 81.6 (C-1), 68.4 (C-2), 70.3 (C-3), 67.6 (C-4), 71.4 (C-5), 61.1 (C-6), 125.0 (C-1′), 122.5 (C-2′), 147.2 (C-3′), 149.6 (C-4′), 115.5 (C-5′), 111.1 (C-6′), 144.4 (C-imine), 20.3–20.5 (CH_3CO), 169.3–170.5 (CH_3CO), 63.93 [3′-OCH_2CH$_3$], 14.68 [3′-OCH$_2$$CH_3$]; ESI-MS *m*/*z*: 570 (M + H, 100%), 592 (M + Na, 87%). Anal. Calcd for $C_{24}H_{31}N_3O_{11}S$ (569.58): C, 50.61; H, 5.49; N, 7.38%. Found: C, 50.70; H, 5.54; N, 7.49%.

*4.2.2.3. Synthesis of 3-methoxy-4-hydroxybenzaldehyde N-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)thiosemicarbazone (**4c**).* White solid, mp 246–247 °C (from 96% ethanol); $[\alpha]_D^{25}$ −87 (*c* 1.8, $CHCl_3$); IR (KBr, cm^{-1}): ν 3352 (NH), 1744 (C=O), 1601 (CH=N), 1223, 1055; ^{1}H NMR (DMSO-d_6) δ (ppm): 8.51 (d, 1H, J = 8.5 Hz, H-4″), 11.85 (s, 1H, H-2″), 8.01 (s, 1H, H imine), 5.77 (t, 1H, J = 9.0, H-1), 5.26 (t, 1H, J = 9.5 Hz, H-2), 5.42 (dd, 1H, J = 10.0, 3.5, H-3), 5.33 (d, 1H, J = 3.5 Hz, H-4), 4.31 (t, 1H, J = 6.5 Hz, H-5), 4.05 (m, 1H, H-6), 7.48 (d, 1H, J = 1.5 Hz, H-2′), 6.83 (d, 1H, J = 8.0 Hz, H-5′), 7.12 (dd, J = 8.0, 4.0 Hz, H-6′), 1.96–2.14 (s, 1H, CH_3CO); ^{13}C NMR (DMSO-d_6) δ (ppm): 177.9 (C=S), 81.5 (C-1), 68.4 (C-2), 70.3 (C-3), 67.6 (C-4), 71.4 (C-5), 61.1 (C-6), 125.1 (C-1′), 109.6 (C-2′), 148.1 (C-3′), 149.2 (C-4′), 119.3 (C-5′), 122.6 (C-6′), 144.3 (C-imine), 20.3–20.5 (CH_3CO), 169.3–170.5 (CH_3CO), 55.7 (3′-OCH$_3$); ESI-MS *m*/*z*: 556 (M + H, 65%), 578 (M + Na, 100%). Anal. Calcd for $C_{23}H_{29}N_3O_{11}S$ (555.55): C, 49.72; H, 5.26; N, 7.56%. Found: C, 49.85; H, 5.38; N, 7.67%.

*4.2.2.4. Synthesis of 3-hydroxy-4-methoxybenzaldehyde N-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)thiosemicarbazone (**4d**).* White solid, mp 181–182 °C (from 96% ethanol); $[\alpha]_D^{25}$ −117 (*c* 2.0, $CHCl_3$); IR (KBr, cm^{-1}): ν 3313 (NH), 1744 (C=O), 1600 (CH=N), 1243, 1040 (C–O–C); ^{1}H NMR (DMSO-d_6) δ (ppm): 8.51 (d, 1H, J = 9.0 Hz, H-4″), 11.78 (s, 1H, H-2″), 7.98 (s, 1H, H imine), 5.89 (t, 1H, J = 9.0 Hz, H-1), 5.26 (t, 1H,

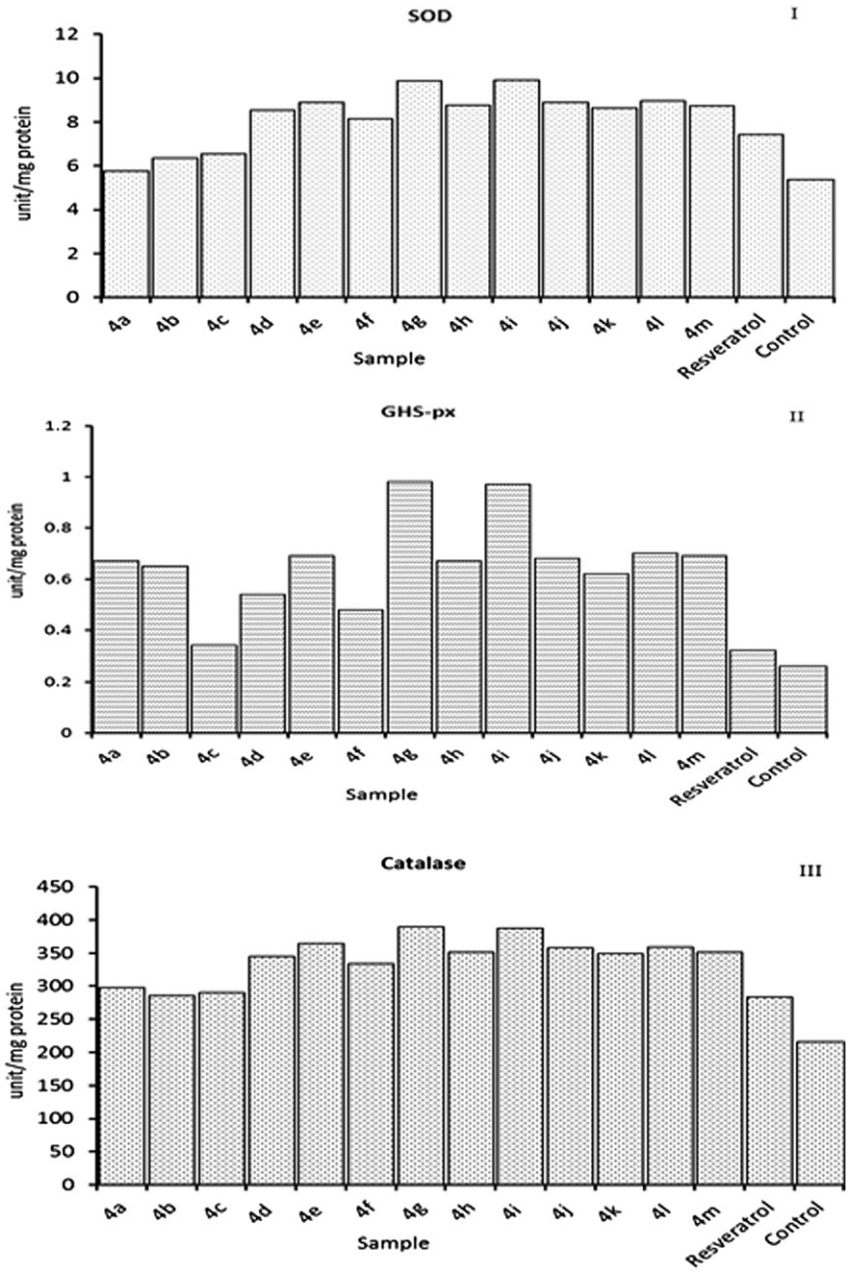


Fig. 4. Effect of compounds **4a**–**m** on the liver cytosolic sod, the liver cytosolic GSH-Px, the liver cytosolic catalase activities and the hepatic MDA production.

J = 9.5 Hz, H-2), 5.39 (dd, 1H, J = 10.0, 4.0 Hz, H-3), 5.32 (d, 1H, J = 3.5 Hz, H-4), 4.31 (t, 1H, J = 6.5 Hz, H-5), 4.04 (d, 1H, J = 6.5 Hz, H-6), 7.31 (d, 1H, J = 2.0 Hz, H-2′), 6.96 (d, 1H, J = 8.5 Hz, H-5′), 7.14 (dd, 1H, J = 8.5, 2.0 Hz, H-6′), 1.93–2.15 (s, 1H, CH_3CO); ^{13}C NMR (DMSO-d_6) δ (ppm): 177.8 (C═S), 81.7 (C-1), 68.6 (C-2), 70.5 (C-3), 67.5 (C-4), 71.6 (C-5), 61.3 (C-6), 126.5 (C-1′), 120.7 (C-2′), 146.7 (C-3′), 150.0 (C-4′), 113.3 (C-5′), 111.8 (C-6′), 144.5 (C-imine), 20.3–20.5 (CH_3CO), 169.3–170.0 (CH_3CO), 55.69 (4′-OCH_3); ESI-MS m/z: 556 (M + H, 36%), 578 (M + Na, 100%). Anal. Calcd for $C_{23}H_{29}N_3O_{11}S$ (555.55): C, 49.72; H, 5.26; N, 7.56%. Found: C, 49.87; H, 5.43; N, 7.69%.

*4.2.2.5. Synthesis of 3-methoxybenzaldehyde N-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)thiosemicarbazone (**4e**).* White solid, mp 223–224 °C (from 96% ethanol); $[\alpha]_D^{25}$ −96 (c 2.5, $CHCl_3$); IR (KBr, cm^{-1}): ν 3348 (NH), 1745 (C═O), 1582 (CH═N), 1220, 1055 (C–O–C); ^1H NMR (DMSO-d_6) δ (ppm): 8.67 (d, 1H, J = 8.5 Hz, H-4″), 11.97 (s, 1H, H-2″), 8.08 (s, 1H, H imine), 5.82 (t, 1H, J = 9.0 Hz, H-1), 5.29 (t, 1H, J = 10.0 Hz, H-2), 5.40 (dd, 1H, J = 10.0, 4.0 Hz, H-3), 5.33 (d, 1H, J = 3.5 Hz, H-4), 4.31 (t, 1H, J = 6.5 Hz, H-5), 4.05 (m, 1H, H-6), 7.46 (d, 1H, J = 1.0 Hz, H-2′), 7.34 (m, 1H, H-4′), 7.34 (m, 1H, H-5′), 7.01 (ddd, 1H, J = 8.0, 1.4, 1.0 Hz, H-6′), 1.95–2.14 (s, 1H, CH_3CO); ^{13}C NMR (DMSO-d_6) δ (ppm): 178.4 (C═S), 81.6 (C-1), 68.5 (C-2), 70.4 (C-3), 67.5 (C-4), 71.5 (C-5), 61.2 (C-6), 135.1 (C-1′), 129.8 (C-2′), 159.6 (C-3′), 120.8 (C-4′), 111.4 (C-5′), 116.6 (C-6′), 143.7 (C-imine), 20.3–20.5 (CH_3CO), 169.3–170.3 (CH_3CO), 55.3 (s, 3H, 3′-OCH_3); ESI-MS m/z: 540 (M + H, 100%), 562 (M + Na, 83%). Anal. Calcd for $C_{23}H_{29}N_3O_{10}S$ (539.56): C, 51.20; H, 5.42; N, 7.79%. Found: C, 51.38; H, 5.57; N, 7.97%.

*4.2.2.6. Synthesis of 4-hydroxybenzaldehyde N-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)thiosemicarbazone (**4f**).* White solid, mp 234–235 °C (from 96% ethanol); $[\alpha]_D^{25}$ −102 (c 2.0, $CHCl_3$); IR (KBr, cm^{-1}): ν 3354 (NH), 1752 (C═O), 1608 (CH═N), 1216, 1039 (C–O–C);

^{1}H NMR (DMSO-d_6) δ (ppm): 8.53 (d, 1H, J = 9.0 Hz, H-4″), 11.76 (s, 1H, H-2″), 8.01 (s, 1H, H imine), 5.86 (t, 1H, J = 9.0 Hz, H-1), 5.23 (t, 1H, J = 9.5 Hz, H-2), 5.38 (dd, J = 10.0, 4.0 Hz, H-3), 5.33 (d, 1H, J = 3.5 Hz, H-4), 4.30 (t, 1H, J = 6.0 Hz, H-5), 4.04 (d, 1H, J = 7.0 Hz, H-6), 6.82 (d, 1H, J = 8.5 Hz, H-2′), 7.65 (d, 1H, J = 8.5 Hz, H-3′), 7.65 (d, 1H, J = 8.5 Hz, H-5′), 6.82 (d, 1H, J = 8.5 Hz, H-6′), 1.94–2.14 (s, 1H, CH_3CO); ^{13}C NMR (DMSO-d_6) δ (ppm): 177.8 (C=S), 81.6 (C-1), 68.6 (C-2), 70.5 (C-3), 67.5 (C-4), 71.5 (C-5), 61.3 (C-6), 144.3 (C-1′), 129.4 (C-2′), 115.7 (C-3′), 124.7 (C-4′), 115.7 (C-5′), 129.4 (C-6′), 159.7 (C-imine), 20.3–20.5 (CH_3CO), 169.4–170.1 (CH_3CO); ESI-MS m/z: 526 (M + H, 81%), 548 (M + Na, 100%). Anal. Calcd for $C_{22}H_{27}N_3O_{10}S$ (525.53): C, 50.28; H, 5.18; N, 8.00%. Found: C, 50.35; H, 5.37; N, 8.19%.

*4.2.2.7. Synthesis of 4-isopropylbenzaldehyde N-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)thiosemicarbazone (**4g**).* White solid, mp 172–173 °C (from 96% ethanol); $[\alpha]_D^{25}$ −100 (c 1.8, $CHCl_3$); IR (KBr, cm^{-1}): ν 3355 (NH), 1748 (C=O), 1608 (CH=N), 1223, 1054 (C–O–C); ^{1}H NMR (DMSO-d_6) δ (ppm): 8.63 (d, 1H, J = 9.5 Hz, H-4″), 11.92 (s, 1H, H-2″), 8.10 (s, 1H, H imine), 5.87 (t, 1H, J = 9.5 Hz, H-1), 5.30 (t, 1H, J = 10.0 Hz, H-2), 5.41 (dd, 1H, J = 10.0, 3.5 Hz, H-3), 5.35 (d, 1H, J = 3.5 Hz, H-4), 4.33 (t, 1H, J = 6.5 Hz, H-5), 4.06 (d, 1H, J = 6.5 Hz, H-6), 7.32 (d, 1H, J = 8.0 Hz, H-2′), 7.50 (d, 1H, J = 8.0 Hz, H-3′), 7.50 (d, 1H, J = 8.0 Hz, H-5′), 7.32 (d, 1H, J = 8.0 Hz, H-6′), 1.96–2.16 (s, 1H, CH_3CO); ^{13}C NMR (DMSO-d_6) δ (ppm): 178.2 (C=S), 81.6 (C-1), 68.5 (C-2), 70.5 (C-3), 67.5 (C-4), 71.5 (C-5), 61.2 (C-6), 131.4 (C-1′), 126.6 (C-2′), 127.6 (C-3′), 151.0 (C-4′), 127.6 (C-5′), 126.6 (C-6′), 143.9 (C-imine), 20.3–20.5 (CH_3CO), 169.3–170.0 (CH_3CO), 33.3 [4′-CH(CH_3)$_2$], 23.6 [4′-CH(CH_3)$_2$]; ESI-MS m/z: 552 (M + H, 88%), 574 (M + Na, 100%). Anal. Calcd for $C_{25}H_{33}N_3O_9S$ (525.53): C, 54.43; H, 6.03; N, 7.62%. Found: C, 54.61; H, 6.24; N, 7.81%.

*4.2.2.8. Synthesis of 4-methybenzaldehyde N-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)thiosemicarbazone (**4h**).* White solid, mp 180–181 °C (from 96% ethanol); $[\alpha]_D^{25}$ −115 (c 2.0, $CHCl_3$); IR (KBr, cm^{-1}): ν 3334 (NH), 1747 (C=O), 1609 (CH=N), 1233, 1054 (C–O–C); ^{1}H NMR (DMSO-d_6) δ (ppm): 8.62 (d, 1H, J = 9.0 Hz, H-4″), 11.85 (s, 1H, H-2″), 8.06 (s, 1H, H imine), 5.85 (t, 1H, J = 9.5 HZ, H-1), 5.27 (t, 1H, J = 10.0 Hz, H-2), 5.36 (dd, 1H, J = 9.5, 4.0 Hz, H-3), 5.31 (d, 1H, J = 3.5 Hz, H-4), 4.29 (t, 1H, J = 6.5 Hz, H-5), 4.03 (d, 1H, J = 6.5 Hz, H-6), 7.69 (d, 1H, J = 8.0 Hz, H-2′), 7.23 (d, 1H, J = 8.0 Hz, H-3′), 7.23 (d, 1H, J = 8.0 Hz, H-5′), 7.69 (d, 1H, J = 8.0 Hz, H-6′), 1.93–2.13 (s, 12H, CH_3CO); ^{13}C NMR (DMSO-d_6) δ (ppm): 178.2 (C=S), 81.8 (C-1), 68.6 (C-2), 70.6 (C-3), 67.6 (C-4), 71.6 (C-5), 61.3 (C-6), 131.0 (C-1′), 129.4 (C-2′), 127.6 (C-3′), 140.3 (C-4′), 127.6 (C-5′), 129.4 (C-6′), 144.1 (C-imine), 20.4–21.0 (CH_3CO), 169.4–170.1 (CH_3CO), 18.5 (4′-CH_3); ESI-MS m/z: 524 (M + H, 100%), 546 (M + Na, 84%). Anal. Calcd for $C_{23}H_{29}N_3O_9S$ (523.56): C, 52.76; H, 5.58; N, 8.03%. Found: C, 52.96; H, 5.75; N, 8.22%.

*4.2.2.9. Synthesis of 4-bromobenzaldehyde N-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)thiosemicarbazone (**4i**).* White solid, mp 159–160 °C (from 96% ethanol); $[\alpha]_D^{25}$ −115 (c 2.0, $CHCl_3$); IR (KBr, cm^{-1}): ν 3331 (NH), 1748 (C=O), 1595 (CH=N), 1227, 1052 (C–O–C); ^{1}H NMR (DMSO-d_6) δ (ppm): 8.77 (d, 1H, J = 9.0 Hz, H-4″), 11.95 (s, 1H, H-2″), 8.06 (s, 1H, H imine), 5.88 (t, 1H, J = 9.0 Hz, H-1), 5.30 (t, 1H, J = 10.0 Hz, H-2), 5.37 (dd, 1H, J = 10.0, 4.0 Hz, H-3), 5.31 (d, 1H, 4.5, H-4), 4.30 (t, 1H, J = 6.5 Hz, H-5), 4.03 (d, 1H, J = 6.5 Hz, H-6), 7.79 (d, 1H, J = 8.5 Hz, H-2′), 7.61 (d, 1H, J = 8.5 Hz, H-3′), 7.61 (d, 1H, J = 8.5 Hz, H-5′), 7.79 (d, 1H, J = 8.5 Hz, H-6′), 1.93–2.13 (s, 12H, CH_3CO); ^{13}C NMR (DMSO-d_6) δ (ppm): 178.4 (C=S), 81.8 (C-1), 68.6 (C-2), 70.5 (C-3), 67.5 (C-4), 71.6 (C-5), 61.2 (C-6), 133.1 (C-1′), 131.6 (C-2′), 129.4 (C-3′), 123.5 (C-4′), 129.4 (C-5′), 131.6 (C-6′), 142.6 (C-imine), 20.3–20.5 (CH_3CO), 169.3–169.9 (CH_3CO); ESI-MS m/z: 588/590 (M + H, 89%/78%), 610/612 (M + Na, 100%/97%). Anal. Calcd for $C_{22}H_{26}BrN_3O_9S$ (588.43): C, 44.91; H, 4.45; N, 7.14%. Found: C, 45.09; H, 4.65; N, 7.32%.

*4.2.2.10. Synthesis of 4-chlorobenzaldehyde N-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)thiosemicarbazone (**4j**).* White solid, mp 173–174 °C (from 96% ethanol); $[\alpha]_D^{25}$ −112 (c 2.0, $CHCl_3$); IR (KBr, cm^{-1}): ν 3325 (NH), 1754 (C=O), 1600 (CH=N), 1245, 1054 (C–O–C); ^{1}H NMR (DMSO-d_6) δ (ppm): 8.78 (d, 1H, J = 9.0 Hz, H-4″), 11.95 (s, 1H, H-2″), 8.08 (s, 1H, H imine), 5.88 (t, 1H, J = 9.0 Hz, H-1), 5.30 (t, 1H, J = 9.5 Hz, H-2), 5.37 (dd, 1H, J = 10, 3.5 Hz, H-3), 5.32 (d, 1H, J = 4.0 Hz, H-4), 4.30 (t, 1H, J = 6.5 Hz, H-5), 4.04 (d, 1H, J = 6.5 Hz, H-6), 7.48 (d, 1H, J = 8.5 Hz, H-2′), 7.86 (d, 1H, J = 8.5 Hz, H-3′), 7.86 (d, 1H, J = 8.5 Hz, H-5′), 7.48 (d, 1H, 8.5 Hz, H-6′), 2.02–2.15 (s, 12H, CH_3CO); ^{13}C NMR (DMSO-d_6) δ (ppm): 178.5 (C=S), 81.9 (C-1), 68.7 (C-2), 70.7 (C-3), 67.6 (C-4), 71.7 (C-5), 61.4 (C-6), 134.9 (C-1′), 128.9 (C-2′), 129.4 (C-3′), 132.8 (C-4′), 129.4 (C-5′), 128.9 (C-6′), 142.7 (C-imine), 20.4–20.6 (CH_3CO), 169.5–170.2 (CH_3CO); ESI-MS m/z: 544/546 (M + H, 100%/34%), 566/568 (M + Na, 98%/39%). Anal. Calcd for $C_{22}H_{26}ClN_3O_9S$ (543.97): C, 48.57; H, 4.82; N, 7.72%. Found: C, 48.77; H, 5.00; N, 7.91%.

*4.2.2.11. Synthesis of 4-fluorobenzaldehyde N-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)thiosemicarbazone (**4k**).* White solid; mp 113–114 °C (from 96% ethanol); $[\alpha]_D^{25}$ −95 (c 2.0, $CHCl_3$); IR (KBr, cm^{-1}): ν 3341 (NH), 1606 (CH=N), 1750 (C=O), 1261, 1045 (C–O–C); ^{1}H NMR (DMSO-d_6) δ (ppm): 8.75 (d, 1H, J = 9.0 Hz, H-4″), 11.93 (s, 1H, H-2″), 8.11 (s, 1H, H imine), 5.90 (t, 1H, J = 9.0 Hz, H-1), 5.32 (m, 1H, H-2), 5.40 (dd, 1H, J = 10.0, 3.5 Hz, H-3), 5.32 (m, 1H, H-4), 4.33 (t, 1H, J = 6.0 Hz, H-5), 4.06 (m, 1H, H-6), 7.28 (t, 1H, J = 9.0 Hz, H-2′), 7.92 (dd, 1H, J = 9.0, 6.0 Hz, H-3′), 7.92 (dd, 9.0, 6.0 Hz, H-5′), 7.28 (t, 1H, J = 9.0 Hz, H-6′), 2.02–2.15 (s, 12H, CH_3CO); ^{13}C NMR (DMSO-d_6) δ (ppm): 178.4 (C=S), 81.8 (C-1), 68.6 (C-2), 70.6 (C-3), 67.5 (C-4), 71.6 (C-5), 61.2 (C-6), 130.4 (C-1′), 129.8 (C-2′), 115.7 (C-3′), 163.3 (C-4′), 115.7 (C-5′), 129.8 (C-6′), 142.7 (C-imine), 20.3–20.5 (CH_3CO), 169.3–170.0 (CH_3CO); ESI-MS m/z: 528 (M + H, 66%), 550 (M + Na, 100%). Anal. Calcd for $C_{22}H_{26}FN_3O_9S$ (543.97): C, 50.09; H, 4.97; N, 7.97%. Found: C, 50.18; H, 5.15; N, 7.81%.

*4.2.2.12. Synthesis of 3-nitrobenzaldehyde N-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)thiosemicarbazone (**4l**).* Light yellow solid; mp 169–170 °C (from 96% ethanol); $[\alpha]_D^{25}$ −98 (c 2.0, $CHCl_3$); IR (KBr, cm^{-1}): ν 3338 (NH), 1745 (C=O), 1625 (CH=N), 1228, 1054 (C–O–C); ^{1}H NMR (DMSO-d_6) δ (ppm): 8.96 (d, 1H, 1H, J = 9.0 Hz, H-4″), 12.13 (s, 1H, 1H, H-2″), 8.22 (s, 1H, 1H, H imine), 5.91 (t, 1H, J = 9.0 Hz, H-1), 5.34 (m, 1H, 1H, H-2), 5.41 (dd, 1H, J = 9.5, 3.5 Hz, H-3), 5.34 (m, 1H, 1H, H-4), 4.34 (t, 1H, J = 6.5 Hz, H-5), 4.06 (m, 1H, H-6), 8.22 (s, 1H, H-2′), 8.36 (d, 1H, J = 8.0 Hz, H-4′), 7.74 (t, 1H, J = 8.0 Hz, H-5′), 8.26 (dd, 1H, J = 8.0, 1.0 Hz, H-6′), 1.96–2.00 (s, 1H, CH_3CO); ^{13}C NMR (DMSO-d_6) δ (ppm): 178.7 (C=S), 81.89 (C-1), 68.6 (C-2), 70.5 (C-3), 67.5 (C-4), 71.6 (C-5), 61.2 (C-6), 130.2 (C-1′), 135.7 (C-2′), 141.6 (C-3′), 133.4 (C-4′), 124.4 (C-5′), 122.1 (C-6′), 148.3 (C-imine), 20.3–20.5 (CH_3CO), 169.3–170.0 (CH_3CO); ESI-MS m/z: 554 (M^+, 100%). Anal. Calcd for $C_{22}H_{26}N_4O_{11}S$ (543.97): C, 47.65; H, 4.73; N, 10.10%. Found: C, 47.84; H, 4.91; N, 10.29%.

*4.2.2.13. Synthesis of 4-nitrobenzaldehyde N-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)thiosemicarbazone (**4m**).* Light yellow solid; mp 157–158 °C (from 96% ethanol); $[\alpha]_D^{25}$ −95 (c 2.0, $CHCl_3$); IR (KBr, cm^{-1}): ν 3337 (NH), 1744 (C=O), 1587 (CH=N), 1226, 1048 (C–O–C); ^{1}H NMR (DMSO-d_6) δ (ppm): 9.00 (d, 1H, 1H, J = 9.0 Hz, H-4″), 12.17 (s, 1H, 1H, H-2″), 8.20 (s, 1H, 1H, H imine), 5.93 (t, 1H, J = 9.0 Hz, H-1), 5.35 (m, 1H, 1H, H-2), 5.40 (dd, 1H, J = 10.0, 3.5 Hz, H-3), 5.35 (m, 1H, 1H, H-4), 4.33 (t, 1H, J = 6.5 Hz, H-5), 4.07 (d, 1H, 1H, J = 6.5 Hz, H-6), 8.14 (d, 1H, 1H, J = 9.0 Hz, H-2′), 8.27 (d, 1H, 1H, J = 9.0 Hz, H-3′), 8.27 (d, 1H, 1H, J = 9.0 Hz, H-5′), 8.14 (d, 1H, 1H,

J = 9.0 Hz, H-6′), 1.96–2.16 (s, 1H, 12H, CH_3CO); ^{13}C NMR (DMSO-d_6) δ (ppm): 178.8 (C=S), 81.9 (C-1), 68.7 (C-2), 70.6 (C-3), 67.5 (C-4), 71.7 (C-5), 61.3 (C-6), 140.2 (C-1′), 123.8 (C-2′), 128.5 (C-3′), 141.2 (C-4′), 128.5 (C-5′), 123.8 (C-6′), 147.9 (C-imine), 20.3–20.5 (CH_3CO), 169.4–170.0 (CH_3CO); ESI-MS m/z: 555 (M + H, 72%), 577 (M + Na, 100%). Anal. Calcd for $C_{22}H_{26}N_4O_{11}S$ (543.97): C, 47.65; H, 4.73; N, 10.10%. Found: C, 47.85; H, 4.93; N, 10.27%.

4.3. Screening for antioxidant activity

4.3.1. Chemicals

Chrysin, dicyclohexylcarbodiimide (DCC) and diethylphosphoryl cyanide (DEPC) were purchased from Sigma Chemical Co. Other derivatizing reagents were obtained from Aldrich Chemical Co. Sodium azide, ethylenediaminetetraacetic acid (EDTA), β-nicotinamide adenine dinucleotide phosphate, reduced form (NADPH), cumene hydroperoxide, glutathione reductase, DL-α-tocopherol acetate, carbon tetrachloride (CCl_4), xanthine, potassium cyanide (KCN), sodium dodecylsulfate, trichloroacetic acid (TCA), cytochrome C, thiobarbituric acid, *n*-butanol and pyridine were purchased from Sigma Chem. Co. All other chemicals and reagents were analytical grade.

4.3.2. Screening for antioxidant activity by DPPH method

All the synthesized compounds were evaluated for antioxidant activity and compared with standard drug (Resveratrol). The activity was evaluated using the DPPH method [36–38]. The 150-mM solution of DPPH (195 μl) in 96% ethanol was added to standard solution (resveratrol) and tested sample solutions (5 μl each) of different concentrations (0.5, 1.0, 2.0, 4.0, 8.0 and 12.0 mM) in 96% ethanol on 96-hole ELISA plates and allow to react at temperature 25 °C in incubator. After 30 min the absorbance values were measured at 518 nm and convert into the percentage antioxidant activity (AA) using formula, $AA\% = [(Abs_{DPPH} - Abs_{sample})/(Abs_{DPPH} - Abs_{ethanol})] \cdot 100\%$, where Abs_{DPPH} was the absorbance of DPPH solution which was used as a negative sample, prepared by adding 96% ethanol (5 μl) to 195 μl of 150-mM solution of DPPH in 96% ethanol, Abs_{sample} was the absorbance of sample solution, $Abs_{ethanol}$ was the absorbance of 96% ethanol, which was used as a blank [38,39]. The positive controls were those using the standard solution containing resveratrol. All tests and analyses were undertaken on three replicates and the results averaged. The IC_{50} values were calculated by linear regression plots, where the abscissa represented the concentration of tested compound solution (0.5, 1.0, 2.0, 4.0, 8.0 and 12.0 mM) and the ordinate the average percent of antioxidant activity from three separate tests. The results are tabulated in Table 2.

4.3.3. Anti-oxidant assay in vivo

Albino rats of Wistar strain, weighing 100–150 g were used in all experiments. Animals were maintained on 12 h light/dark cycle at approximately 22 °C and allowed food and water ad libitum. Rats were injected i.p., with a mixture of CCl_4 in olive oil (1:1) at a dose of 0.6 mL/kg to induce hepatotoxicity. These animals were randomized into four groups and seven rats each. Control animals were given the vehicle alone. Rats were pretreated once with DL-α-tocopherol acetate (a dose of 400 mg/kg) and test samples were given i.p. at a dose of 100 mg/kg/day for seven consecutive days prior to the administration of CCl_4. Animals were sacrificed 24 h after CCl_4 dosing and blood was collected by decapitation for the determination of serum transaminases.

Hepatic tissues were carefully excised and homogenized in cold 1.15% KCl–10 mM phosphate buffer with EDTA (pH 7.4) and centrifuged at 12,000 rpm for 8 min. The supernatant was further centrifuged at 45,000 rpm for 50 min to obtain cytosolic extract for the measurement of liver cytosolic SOD, catalase and GSH-Px activities. The protein content was measured by the method of Lowry et al. [45] with bovine serum albumin as a standard.

4.3.4. Determination of anti-oxidant enzyme activities

SOD was assayed by the method of McCord and Fridovich [46]. The reaction mixture was make from 300 μl of 0.5 mM solution of xanthine as substrate, 100 μl of 0.05 mM solution of KCN, 100 μl of solution of 1% sodium deoxycholate, 20 μl of solution of xanthine oxidase, 20 μl of solution of cytosolic extract and 300 μl of solution of 0.1 mM cytochrome C and placed in a 1 cm cuvette and the rate of increase in absorbance at 550 nm was recorded for 5 min. SOD activity was expressed as unit/mg protein (Table 3).

Catalase was assayed by the method of Rigo and Rotilio [47]. The cytosolic extract of liver (40 μl) diluted 10 times was added with 0.13 mM phosphate buffer (pH 7.0, 500 μl), distilled by 660 μl of water and 1800 μl of 15 mM solution of H_2O_2 and thoroughly mixed. The rate of changes in the absorbance at 240 nm for 5 min was recorded. Catalase activity was expressed as unit/mg protein (Table 3).

Glutathione peroxidase (GSH-Px) activity was measured by the method of Paglia and Valentine [48]. The enzymatic reaction in the tube that contained reduced nicotinamide adenine dinucleotide phosphate, reduced glutathione, sodium azide and glutathione reductase was initiated by the addition of hydrogen peroxide (H_2O_2) and the change in absorbance at 340 nm was monitored by a spectrophotometer. Activity was given in units per gram (unit/g) protein (Table 3).

4.3.5. Statistical analysis

All data on antioxidant activities are the average of triplicate analyses. One-way analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Duncan's Multiple Range tests. P values < 0.05 were regarded as significant and P values < 0.01 were very significant [36].

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Appendix A. Supplementary data

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References

[1] Z.J. Witczak, Monosaccharide isothiocyanates and thiocyanates: synthesis, chemistry, and preparative applications, in: S. Tipson (Ed.), Advances in Carbohydrate Chemistry and Biochemistry, vol. 44, Academic Press, New York, 1986, pp. 91–145 (Chapter 3).
[2] J.L. Jiménez Blanco, B. Sylla, C. Ortiz-Mellet, J.M. García Fernández, J. Org. Chem. 72 (2007) 4547–4550.
[3] D.C. Greenbaum, Z. Mackey, E. Hansell, P. Doyle, J. Gut, C.R. Caffrey, J. Lehrman, P.J. Rosenthal, J.H. McKerrow, K. Chibale, J. Med. Chem. 47 (2004) 3212–3219.
[4] B. Yang, S.S. Zhang, H.X. Li, Chem. Res. Chin. Univ. 22 (2006) 738–741.
[5] M.A.M. Alho, N.B. d'Accorso, Carbohydr. Res. 328 (2000) 481–488.
[6] M.R. Ganjali, M. Hosseini, M. Salavati-Niasari, T. Poursaberi, M. Shamsipur, M. Javanbakht, O.R. Hashemi, Electroanalysis 14 (2002) 526–531.
[7] A.D. Naik, P.A.N. Reddy, M. Nethaji, A.R. Chakravarty, Inorg. Chim. Acta 349 (2003) 149–158.
[8] N.M. El-Metwally, I.M. Gabr, A.M. Shallaby, A.A. El-Asmy, J. Coord. Chem. 58 (2005) 1145–1149.
[9] S. Sharma, F. Athar, M.R. Maurya, A. Azam, Eur. J. Med. Chem. 40 (2005) 1414–1419.
[10] L.S. Sarma, J.R. Kumar, K.J. Reddy, A.V. Reddy, J. Agric. Food Chem. 53 (2005) 5492–5498.
[11] M.M. Aly, Y.A. Mohamed, K.A.M. El-Bayouki, W.M. Basyouni, S.Y. Abbas, Eur. J. Med. Chem. 45 (2010) 3365–3373.

[12] T.P. Stanojkovic, D. Kovala-Demertzi, A. Primikyri, I. Garcia-Santos, A. Castineiras, Z. Juranic, M.A. Demertzis, J. Inorg. Biochem. 104 (2010) 467–476.
[13] Zeng-Chen Liu, Bao-Dui Wang, Zheng-Yin Yang, Yong Li, Dong-Dong Qin, Tian-Rong Li, Eur. J. Med. Chem. 44 (2009) 4477–4484.
[14] D. Kovala-Demertzi, P.N. Yadav, J. Wiecek, S. Skoulika, T. Varadinova, M.A. Demertzis, J. Inorg. Biochem. 100 (2006) 1558–1567.
[15] I.D. Kostas, G.A. Heropoulos, D. Kovala-Demertzi, P.N. Yadav, J.P. Jasinski, M.A. Demertzis, F.J. Andreadaki, G. Vo-Thanh, A. Petit, A. Loupy, Tetrahedron Lett. 47 (2006) 4403–4407.
[16] D. Sriram, P. Yogeeswari, R. Thirumurugan, R.K. Pavana, J. Med. Chem. 49 (2006) 3448–3450.
[17] N.C. Desai, H.K. Shucla, B.R. Parekh, K.A. Thaker, J. Indian Chem. Soc. 61 (1984) 455–457.
[18] A.P. Liesen, T.M. Aquino, C.S. Carvalho, V.T. Lima, J.M. Araújo, J.G. Lima, Antônio R. de Faria, Edésio J.T. de Melo, Antonio J. Alves, Elias W. Alves, A.Q. Alves, A.J.S. Góes, Eur. J. Med. Chem. 45 (2010) 3685–3691.
[19] M.G. Mamolo, L. Vio, E. Banfi, Farmaco 51 (1996) 71–74.
[20] B.K. Garnaik, R.K. Behera, Indian J. Chem. 27B (1988) 1157–1158.
[21] L. Labanauskas, V. Kalcas, E. Udrenaite, P. Gaidelis, A. Brukstus, V. Dauksas, Pharmazie 56 (2001) 617–619.
[22] L. Somogyi, Carbohydr. Res. 75 (1979) 325–330.
[23] S. Turner, M. Myers, B. Gadie, S.A. Hale, A. Horsley, A.J. Nelson, R. Pape, J.F. Saville, J.C. Doxey, T.L. Berridge, J. Med. Chem. 31 (1988) 906–913.
[24] G. Mazzone, R. Pignatello, S. Mazzone, A. Panico, G. Pennisi, R. Castana, P. Mazzone, Farmaco 48 (1993) 1207–1224.
[25] E.C. Rodriguez, L.A. Marcaurelle, C.R. Bertozzi, J. Org. Chem. 63 (1998) 7134–7135.
[26] K.N. Zelenin, V.V. Alekseyev, P.B. Terentiev, O.B. Kumetsova, V.V. Lashin, V.V. Ovcharenko, V.V. Torocheshnikov, L.A. Khorseyeva, A.A. Sorokin, Mendeleev. Commun. 3 (1993) 168–169.
[27] J.Y. Chou, S.Y. Lai, S.L. Pan, G.M. Jow, J.W. Chern, J.H. Guh, Biochem. Pharmacol. 66 (2003) 115–124.
[28] M. Hanna, M. Girges, D. Rasala, R. Gawineck, Arzneim. Forsch.-Drug. Res. 45 (1995) 1074–1078.
[29] S. Ghosh, A.K. Misra, G. Bhatia, M.M. Khan, A.K. Khanna, Bioorg. Med. Chem. Lett. 19 (2009) 386–389.
[30] A.C. Tenchiu Deleanu, I.D. Kostas, D. Kovala-Demertzi, A. Terzis, Carbohydr. Res. 344 (2009) 1352–1364.
[31] N.D. Thanh, N.T.T. Mai, Carbohydr. Res. 344 (2009) 2399–2405.
[32] N.D. Thanh, N.T.K. Giang, L.T. Hoai, Eur. J. Chem. 7 (2010) 899–907.
[33] A. Loupy, Microwave in Organic Synthesis, second ed., vol. 1, WILEY-VCH Verlag GmbH & Co KGaA, Weinheim, 2006, pp. 579–594.
[34] A.A. Tashpulatov, V.A. Afanas'ev, M.Yu. Lidak, N.M. Sukhova, Yu.Yu. Popelis, I. Rakhmatullaev, Chem. Heterocycl. Compd. 19 (1983) 137–141.
[35] N. Karalı, A. Gürsoy, F. Kandemirli, N. Shvets, F.B. Kaynak, S. Özbey, V. Kovalishyn, A. Dimoglo, Bioorg. Med. Chem. 15 (2007) 5888–5904.
[36] K. Marxen, K.H. Vanselow, S. Lippemeier, R. Hintze, A. Ruser, U.-P. Hansen, Sensors 7 (2007) 2080–2095.
[37] K. Shimada, K. FuJikawa, K. Yahara, T.J. Nakamura, Agric. Food Chem. 40 (1992) 945–948.
[38] L.P. Leong, G. Shui, Food Chem. 76 (2002) 69–75.
[39] A. Braca, C. Sortino, M. Politi, I. Morelli, J. Mendez, J. Ethnopharmacol. 79 (2002) 379–381.
[40] W. Brand-Williams, M.E. Cuvelier, C. Berset, Lebensm.-Wiss. u.-Technol. 28 (1995) 25–30.
[41] J.M. McCord, I. Fridovich, Free Radic. Biol. Med. 5 (1988) 363–369.
[42] P. Chelikani, I. Fita, P.C. Loewen, Cell. Mol. Life Sci. 61 (2004) 192–208.
[43] F.L. Muller, M.S. Lustgarten, Y. Jang, A. Richardson, H. van Remmen, Free Radic. Biol. Med. 43 (2007) 477–503.
[44] R.L. Lemieux, Tetra-*O*-acetyl-β-D-glucopyranosyl bromide, in: R.L. Whistler, M.L. Wolfom (Eds.), Methods in Carbohydrate Chemistry, vol. 2, Academic Press Inc., New York and London, 1963, pp. 221–222.
[45] O.H. Lowry, N.N. Posenbrough, A.L. Farr, R.J. Randall, J. Biol. Chem. 193 (1951) 265–275.
[46] J.M. McCord, I. Fridovich, J. Biol. Chem. 244 (1969) 6049–6055.
[47] A. Rigo, G. Rotilio, Anal. Biochem. 81 (1977) 157–166.
[48] D.E. Paglia, W.N. Valentine, J. Lab. Clin. Med. 70 (1967) 158–169.