Research Paper

Synthesis, Structure and Antioxidant Activity of (Tetra-*O*-acetyl-β-D-galactopyranosyl)thiosemicarbazones of Substituted Benzaldehydes

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Some new substituted benzaldehyde (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl) thiosemicarbazones were synthesised by reaction of 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl thiosemicarbazide and different substituted benzaldehydes. The reaction was performed using conventional and microwave-assisted heating methods. The structures of thiosemicarbazones were confirmed by spectroscopic (IR, ^{1}H NMR, ^{13}CNMR and 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.

Key words: Antioxidant activity, D-galactose, microwave-assisted, thiosemicarbazones

Thiosemicarbazones, which have NH-C(=S) NHN=C bond, are a class of compounds that have been evaluated over the last 50 years as antivirals and as anticancer therapeutics, as well as for their parasiticidal action against *Plasmodium falciparum* and *Trypanasoma cruzi* which are the causative agents of malaria and Chagas's disease, respectively[1]. The chemistry of thiosemicarbazide derivatives of saccharides is interested[2,3]. These compounds arouse interest as versatile intermediates for preparing various (e.g., heterocyclic) derivatives as well. Thiosemicarbazones can be used for making complex formation of metallic ions[4-13]. Thiosemicarbazones exhibit various biological activities such as antituberculosis[14,15], antimicrobial[9,16-18], antiinflammatory[19], anticonvulsant[9,20], antihypertensive[21], local anesthetic[22], anticancer[10,23], hypoglycemic[24], and cytotoxic activities[9], also antioxidant agents[11,25]. A number of galactosyl thiosemicarbazide derivatives showed significant *in vivo* antimicrobial and *in vitro* antioxidant activity, which could be used as leads for the development of effective antiatherosclerotic agents[2,20,26,27]. 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[25].

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In the past some papers have been published for the synthesis of aldehyde/ketone (per-*O*-acetylated glycopyranosyl)thiosemicarbazones[2,3,18,25,28-30]. The main synthetic step for the synthesis of these molecules is being the reaction of (per-*O*-acetylglycosyl)thiosemicarbazide with the coresponding carbonyl compounds. Continuing our studied on the synthesis and the reactivity of (per-*O*-acetatylglycopyranosyl)isothiocyanate and (per-*O*-acetatylglycopyranosyl) thiosemicarbazides[29,30], we report herein a systematic study for the synthesis and spectral characterization of a series of aromatic aldehyde 4-(β-D-galactopyranosyl)thiosemicarbazones using microwave-assisted method[31].

MATERIALS AND METHODS

All melting points were determined by open capillary method on Stuart SMP3 instrument (Bibby Sterilin Ltd, UK) and are uncorrected. IR spectra (KBr disc) were recorded on a 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. All the starting materials and reagents were purchased from commercial suppliers and used after further purification. (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 (prepared from D-galactose, using the procedure for D-glucose)[32] with lead thiocyanate in dried toluene[18]. (2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)thiosemicarbazide (2) was prepared from corresponding isothiocyanate compound by modifying our method[30].

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

Conventional Method (for compounds 4a, 4b, 4d and 4m): A suspension mixture of (2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)thiosemicarbazide (1) (4.21 g, 1 mmol) and corresponding substituted benzaldehyde 3(a-m) (1 mmol) and glacial acetic acid (1 ml) in methanol (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 benzaldehyde (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)thiosemicarbazones (4a-m).

Microwave-assisted Method (for all compounds): A suspension mixture of (2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)thiosemicarbazide 1 (4.21 g, 1 mmol) and corresponding substituted benzaldehyde 3(a-m) (1 mmol) and glacial acetic acid (0.05 ml) in 99.5% 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 colourless crystals were filtered with suction. The crude product was recrystallized from 95% ethanol or 70% ethanol to afford the title compounds of benzaldehyde (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 MS) data are in good agreement with their structures.

4-Nitrobenzaldehyde (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)thiosemicarbazone (4a):

Light yellow solid; mp 157-158°; IR (KBr, cm^{-1}): 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, *J* 9.0 Hz, H-4"), 12.17 (s, 1H, 1H, H-2"), 8.20 (s, 1H, H imine), 5.93 (t, 1H, *J* 9.0 Hz, H-1), 5.35 (m, 1H, H-2), 5.40 (dd, 1H, *J* 10.0, 3.5 Hz, H-3), 5.35 (m, 1H, H-4), 4.33 (t, 1H, *J* 6.5 Hz, H-5), 4.07 (d, 1H, *J* 6.5 Hz, H-6), 8.14 (d, 1H, *J* 9.0 Hz, H-2'), 8.27 (d, 1H, *J* 9.0 Hz, H-3'), 8.27 (d, 1H, 1H, *J* 9.0 Hz, H-5'), 8.14 (d, 1H, *J* 9.0 Hz, H-6'), 1.96-2.16 (s, 1H, 12H, CH_3CO); ^{13}C NMR (DMSO-d$_6$, δ ppm): 178.84 (C=S), 81.94 (C-1), 68.67 (C-2), 70.61 (C-3), 67.53 (C-4), 71.71 (C-5), 61.28 (C-6), 140.21 (C-1'), 123.77 (C-2'), 128.53 (C-3'), 141.23 (C-4'), 128.53 (C-5'), 123.77 (C-6'), 147.90 (C-imine), 20.32-20.51 (CH_3CO), 169.36-170.01 (CH_3CO); MS *m/z*: 555 (M^+ + H, 72%), 577 (M^+ + Na, 100%) for $C_{22}H_{26}N_4O_{11}S$.

3-Nitrobenzaldehyde (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)thiosemicarbazone (4b):

Light yellow solid; mp 169-170°; IR (KBr, cm^{-1}): 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, *J* 9.0 Hz, H-4"), 12.13 (s, 1H, H-2"), 8.22 (s, 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, 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): 178.69 (C=S), 81.89 (C-1), 68.62 (C-2), 70.50 (C-3), 67.50 (C-4), 71.64 (C-5), 61.23 (C-6), 130.15 (C-1'), 135.71 (C-2'), 141.58 (C-3'), 133.44 (C-4'), 124.40 (C-5'), 122.06 (C-6'), 148.33 (C-imine), 20.32-20.52 (CH_3CO), 169.33-169.99 (CH_3CO); MS *m/z*: 554 (M^+ 100%) for $C_{22}H_{26}N_4O_{11}S$.

4-Fluorobenzaldehyde (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)thiosemicarbazone (4c):

White solid; mp 113-114°; IR (KBr, cm^{-1}): 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, *J* 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): 178.35 (C=S), 81.76 (C-1), 68.61 (C-2), 70.55 (C-3), 67.51 (C-4), 71.56 (C-5), 61.24 (C-6), 130.37 (C-1'), 129.84 (C-2'), 115.73 (C-3'), 163.25 (C-4'), 115.73 (C-5'), 129.84 (C-6'), 142.67 (C-imine), 20.29-20.48 (CH_3CO), 169.31-169.98 (CH_3CO); MS *m/z*: 528 (M^+ + H, 66%), 550 (M^+ + Na, 100%) for $C_{22}H_{26}FN_3O_9S$.

4-Chlorobenzaldehyde (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)thiosemicarbazone (4d):
White solid, mp 173-174°; IR (KBr, cm^{-1}): 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): 178.53 (C=S), 81.92 (C-1), 68.73 (C-2), 70.68 (C-3), 67.62 (C-4), 71.72 (C-5), 61.37 (C-6), 134.86 (C-1'), 128.88 (C-2'), 129.36 (C-3'), 132.81 (C-4'), 129.36 (C-5'), 128.88 (C-6'), 142.70 (C-imine), 20.41-20.61 (CH_3CO), 169.51-170.17 (CH_3CO); MS *m/z*: 544/546 (M^+ + H, 100%/34%), 566/568 (M^+ + Na, 98%/39%) for $C_{22}H_{26}{}^{35}ClN_3O_9S/C_{22}H_{26}{}^{37}ClN_3O_9S$.

4-Bromobenzaldehyde (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)thiosemicarbazone (4e):
White solid, mp 159-160°; IR (KBr, cm^{-1}): 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): 178.41 (C=S), 81.77 (C-1), 68.59 (C-2), 70.54 (C-3), 67.48 (C-4), 71.56 (C-5), 61.21 (C-6), 133.05 (C-1'), 131.62 (C-2'), 129.43 (C-3'), 123.50 (C-4'), 129.43 (C-5'), 131.62 (C-6'), 142.56 (C-imine), 20.28-20.47 (CH_3CO), 169.27-169.94 (CH_3CO); MS *m/z*: 588/590 (M^+ + H, 89%/78%), 610/612 (M^+ + Na, 100%/97%) for $C_{22}H_{26}{}^{79}BrN_3O_9S/C_{22}H_{26}{}^{81}BrN_3O_9S$.

4-Methybenzaldehyde (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)thiosemicarbazone (4f):
White solid, mp 180-181°; IR (KBr, cm^{-1}): 3334 (NH), 1747 (C=), 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): 178.22 (C=S), 81.75 (C-1), 68.63 (C-2), 70.57 (C-3), 67.57 (C-4), 71.59 (C-5), 61.29 (C-6), 131.03 (C-1'), 129.40 (C-2'), 127.62 (C-3'), 140.32 (C-4'), 127.62 (C-5'), 129.40 (C-6'), 144.11 (C-imine), 20.35-21.00 (CH_3CO), 169.41-170.13 (CH_3CO), 18.53 (4'-CH_3); MS *m/z*: 524 (M^+ + H, 100%), 546 (M^+ + Na, 84%) for $C_{23}H_{29}N_3O_9S$.

4-Isopropylbenzaldehyde (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)thiosemicarbazone (4g):
White solid, mp 172-173°; IR (KBr, cm^{-1}): 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): 178.17 (C=S), 81.61 (C-1), 68.53 (C-2), 70.46 (C-3), 67.48 (C-4), 71.48 (C-5), 61.18 (C-6), 131.37 C-1'), 126.64 (C-2'), 127.62 (C-3'), 150.95 (C-4'), 127.62 (C-5'), 126.64 (C-6'), 143.87 (C-imine), 20.26-20.45 (CH_3CO), 169.25-170.02 (CH_3CO), 33.34 [4'-$CH(CH_3)_2$], 23.56 [4'-$CH(CH_3)_2$]; MS *m/z*: 552 (M^+ + H, 88%), 574 (M^+ + Na, 100%) for $C_{25}H_{33}N_3O_9S$.

4-Hydroxybenzaldehyde (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)thiosemicarbazone (4h):
White solid, mp 234-235°; IR (KBr, cm^{-1}): 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): 177.78 (C=S), 81.64 (C-1), 68.61 (C-2), 70.53 (C-3), 67.53 (C-4), 71.51 (C-5), 61.25 (C-6), 144.31 (C-1'), 129.41 (C-2'), 115.66 (C-3'), 124.68 (C-4'), 115.66 (C-5'), 129.41 (C-6'), 159.70 (C-imine),

20.31-20.51 (CH_3CO), 169.35-170.09 (CH_3CO); MS *m/z*: 526 (M^+ + H, 81%), 548 (M^+ + Na, 100%) for $C_{22}H_{27}N_3O_{10}S$.

3-Methoxybenzaldehyde (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)thiosemicarbazone (4i):

White solid, mp 223-224°; IR (KBr, cm^{-1}): 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): 178.42 (C=S), 81.64 (C-1), 68.45 (C-2), 70.41 (C-3), 67.51 (C-4), 71.48 (C-5), 61.16 (C-6), 135.11 (C-1'), 129.78 (C-2'), 159.58 (C-3'), 120.77 (C-4'), 111.38 (C-5'), 116.57 (C-6'), 143.65 (C-imine), 20.32-20.50 (CH_3CO), 169.31-170.25 (CH_3CO), 55.26 (s, 3H, 3'-OCH_3); MS *m/z*: 540 (M^+ + H, 100%), 562 (M^+ + Na, 83%) for $C_{23}H_{29}N_3O_{10}S$.

3-Hydroxy-4-methoxybenzaldehyde (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl) thiosemicarbazone (4j):

White solid, mp 181-182°; IR (KBr, cm^{-1}): 3313 (NH), 1744 (C=O), 1600 (CH=N), 1243, 1040 (C-*O*-C); ^1H 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, *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): 177.79 (C=S), 81.65 (C-1), 68.63 (C-2), 70.53 (C-3), 67.54 (C-4), 71.55 (C-5), 61.29 (C-6), 126.51 (C-1'), 120.70 (C-2'), 146.74 (C-3'), 150.03 (C-4'), 113.31 (C-5'), 111.78 (C-6'), 144.51 (C-imine), 20.33-20.53 (CH_3CO), 169.34-170.04 (CH_3CO), 55.69 (4'-OCH_3); MS *m/z*: 556 (M^+ + H, 36%), 578 (M^+ + Na, 100%) for $C_{23}H_{29}N_3O_{11}S$.

3-Methoxy-4-hydroxybenzaldehyde (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl) thiosemicarbazone (4k):

White solid, mp 246-247°; IR (KBr, cm^{-1}): 3352 (NH), 1744 (C=O), 1601 (CH=N), 1223, 1055; ^1H 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): 177.90 (C=S), 81.54 (C-1), 68.38 (C-2), 70.31 (C-3), 67.55 (C-4), 71.41 (C-5), 61.10 (C-6), 125.07 (C-1'), 109.58 (C-2'), 148.13 (C-3'), 149.23 (C-4'), 119.26 (C-5'), 122.63 (C-6'), 144.28 (C-imine), 20.32-20.49 (CH_3CO), 169.30-170.53 (CH_3CO), 55.73 (3'-OCH_3); MS *m/z*: 556 (M^+ + H, 65%), 578 (M^+ + Na, 100%) for $C_{23}H_{29}N_3O_{11}S$.

3-Ethoxy-4-hydroxybenzaldehyde (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl) thiosemicarbazone (4l):

White solid, mp 204-205°; IR (KBr, cm^{-1}): 3345 (NH), 1747 (C=O), 1600 (CH=N), 1223, 1051 (C-*O*-C); ^1H 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): 177.86 (C=S), 81.56 (C-1), 68.39 (C-2), 70.34 (C-3), 67.56 (C-4), 71.44 (C-5), 61.11 (C-6), 125.03 (C-1'), 122.45 (C-2'), 147.16 (C-3'), 149.56 (C-4'), 115.48 (C-5'), 111.11 (C-6'), 144.44 (C-imine), 20.32-20.48 (CH_3CO), 169.30-170.48 (CH_3CO), 63.93 [3'-OCH_2CH_3], 14.68 [3'-OCH_2CH_3]; MS *m/z*: 570 (M^+ + H, 100%), 592 (M^+ + Na, 87%) for $C_{24}H_{31}N_3O_{11}S$.

4-Dimethylaminobenzaldehyde (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl) thiosemicarbazone (4m):

White solid, mp 217-218°; IR (KBr, cm^{-1}): 3343 (NH), 1744 (C=O), 1600 (CH=N), 1223, 1055 (C-*O*-C); ^1H 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): 177.25 (C=S), 81.50 (C-1), 68.50 (C-2), 70.42 (C-3), 67.48 (C-4), 71.38 (C-5), 61.16 (C-6), 120.77 (C-1'), 111.62 (C-2'), 128.86 (C-3'), 151.65 (C-4'), 128.86 (C-5'), 111.62 (C-6'), 144.80 (C-imine), 20.26-20.45 (CH_3CO), 169.24-170.05 (CH_3CO), 20.37 [4'-$N(CH_3)_2$]; MS *m/z*: 553 (M^+ + H, 100%), 575 (M^+ + Na, 64%) for $C_{24}H_{32}N_4O_9S$.

Screening for Antioxidant activity:

Chrysin, dicyclohexylcarbodiimide (DCC) and diethylphosphoryl cyanide (DEPC) were purchased from Sigma Chemical Co. Other derivatizing reagents were obtained from Aldrich Chemical Co. Sodium azide, ethylenediamine tetraacetic 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.

Screening for Antioxidant activity by DPPH method:

All the synthesised compounds were evaluated for antioxidant activity and comprared with standard drug (resveratrol). The activity was evaluated using the DPPH method[33-35]. The 150mM solution of DPPH (195 µl) 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) on 96-hole ELISA plates and allow to react at temperature 25° in incubator. After 30 min the absorbance values were measured at 518 nm and converted into the percentage antioxidant activity (AA) using formula, $AA\% = [(Abs_{DPPH} - Abs_{sample})/(Abs_{DPPH} - Abs_{ethanol})].100\%$, where Abs_{DPPH} was the absorbance of DPPH solution which was used as a negative prepared by adding 5 µl ethanol to 195 µl of 150 mM solution of DPPH in ethanol, Abs_{sample} was the absorbance of sample solution, $Abs_{ethanol}$ was the absorbance of ethanol, which was used as a blank. 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 1.

Antioxidant 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° 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. 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 sacrified 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*.[36] with bovine serum albumin as a standard.

Determination of antioxidant enzyme activities:

SOD was assayed by the method of McCord and Fridovich[37]. The reaction mixture was make from

TABLE 1: ANTIOXIDANT ACTIVITY OF SYNTHESISED COMPOUNDS BY DPPH METHOD

Conc. Compd.	Scavenging effect for DPPH (%)						IC_{50} (µM)
	12.5	25	50	100	200	300	
4a	6.11	11.32	18.47	29.08	53.30	64.46	210
4b	7.05	13.74	19.63	26.29	38.31	51.24	283
4c	8.51	13.32	17.08	34.34	55.63	67.19	197
4d	7.15	10.09	17.61	19.82	38.37	55.42	270
4e	5.38	9.04	17.46	23.51	35.42	44.31	>300
4f	7.21	12.76	18.06	32.84	53.27	65.03	206
4g	2.17	5.32	9.65	15.09	18.13	24.48	>300
4h	11.45	22.61	33.27	49.18	68.74	75.08	108
4i	7.34	11.46	15.63	27.17	34.02	55.07	276
4j	8.16	17.43	28.21	40.09	56.80	69.61	182
4k	9.45	27.11	45.64	60.30	71.23	74.05	75
4l	14.16	30.24	45.38	59.42	68.34	69.16	71
4m	14.32	30.86	48.94	68.17	74.54	78.47	56
Resveratrol	9.13	22.56	33.84	54.03	70.44	75.62	94

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 soltuion 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.

Catalase was assayed by the method of Rigo and Rotilio[38,39]. 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.

Statistical analysis:

Results were subjected to one-way ANOVA and $p<0.05$ was considered significant. The post hoc analysis was carried out by Dunnet's multiple comparison test[40].

RESULTS AND DISCUSSION

Condensation reaction of tetra-*O*-acetyl-β-D-galactopyranosyl thiosemicarbazide 2 with a number of substituted benzaldehydes 3a-m lead to form a series of benzaldehyde (tetra-*O*-acetyl-b-D-galactopyranosyl)thiosemicarbazones 4a-m (fig. 1 and Table 2). The reaction was performed by using microwave-assissted heating and conventional heating methods. The microwave-assisted synthetic pathway was carried out using minimum amount of solvent

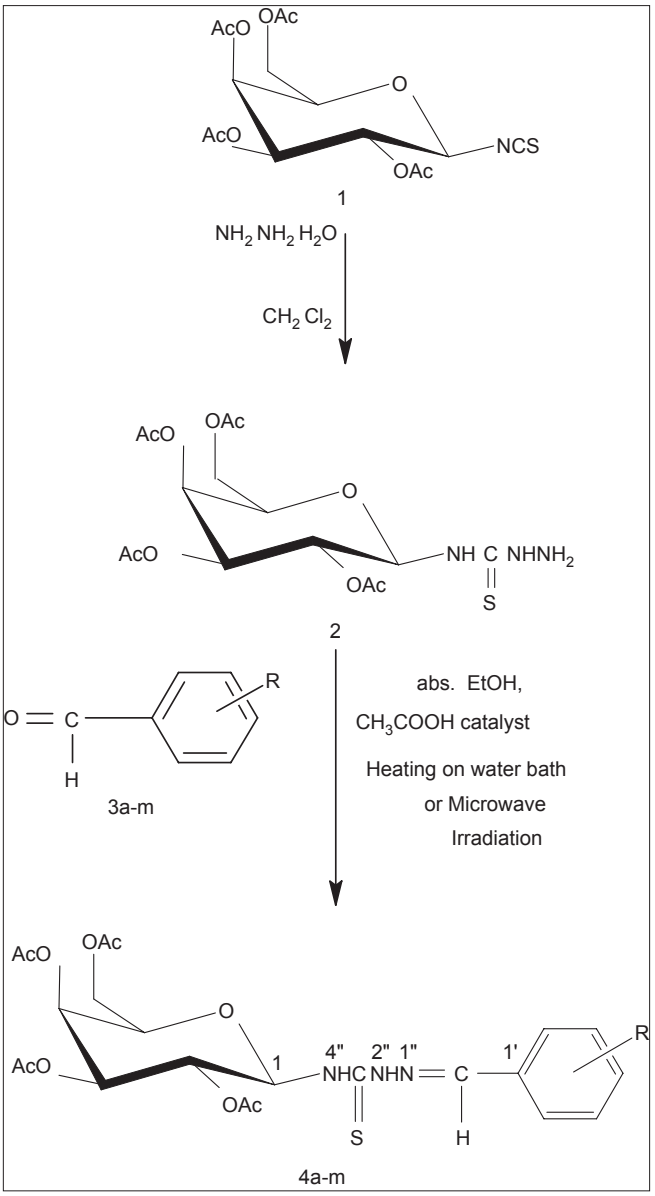

Fig. 1: The synthesis route for preparation of the title compounds 4(a-m).

TABLE 2: 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-NO_2	5	3	97	90	20	48
4b	3-NO_2	5	3	70	90	20	60
4c	4-F	5	2	73			
4d	4-Cl	5	2	98	90	20	32
4e	4-Br	5	2	98			
4f	4-Me	5	2	60			
4g	4-iPr	5	2	75			
4h	4-OH	5	3	75			
4i	3-OMe	5	2	85			
4j	3-OH-4-OMe	7	3	75			
4k	3-OMe-4-OH	7	3	70			
4l	3-OEt-4-OH	7	3	80			
4m	4-NMe_2	7	3	74	90	20	64

(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 min to 7 min depending on substituent's nature: withdrawing substituents need shorter reaction time than donating ones. In the first period of reaction when reaction was starting to irradiate about 1-3 min, the pasty mixture of reagents in methanol was dissolved and the reaction became homogenous. In the final period of reaction the solid product appeared and precipitated out. The products yields of microwawe-asisted 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, DMF,… and have high melting points. The synthesised 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 4(a-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 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 carbonyl groups, respectively. Protons in methyl group of acetate ester had chemical shifts at δ = 2.16-1.93 ppm.

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. 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*.[33], Leong and Shui[34] and Braca *et al*[35].

Amongst the compounds screened for antioxidant activity, 4h, 4k, 4l and 4m showed good antioxidant activity. The compounds with substituents such as 4-OH (4h), 3-OMe-4-OH (4k), 3-OEt-4-OH (4l) and 4-NMe_2 (4m) showed very good antioxidant activity. Remained compounds do not show any antioxidant activity (Table 1, fig. 2 and 3).

Compounds 4a-m were tested *in vivo* for their anti-oxidant acitivities 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

TABLE 3: EFFECT OF COMPOUNDS 4(a-m) ON THE LIVER CYTOSOLIC SOD, THE LIVER CYTOSOLIC GSH-PX, THE LIVER CYTOSOLIC CATALASE ACTIVITIES AND THE HEPATIC MDA PRODUCTION

Compd.	SOD (unit/ mg protein)	GHS-px (unit/ mg protein)	Catalase (unit/ mg protein)
4a	8.75±0.49	0.69±0.02	351.48±12.23
4b	8.96±0.52	0.70±0.01	359.57±11.83
4c	8.65±0.45	0.62±0.01	349.61±12.43
4d	8.89±0.62	0.68±0.01	357.87±12.23
4e	9.90±0.67	0.97±0.01	387.56±12.42
4f	8.78±0.35	0.67±0.02	351.21±11.53
4g	9.89±0.62	0.98±0.01	389.87±12.78
4h	8.14±0.56	0.48±0.02	334.67±10.37
4i	8.91±0.32	0.69±0.01	364.72±11.97
4j	8.54±0.56	0.54±0.02	345.56±11.77
4k	6.54±0.34	0.34±0.03	299.78±13.54
4l	6.35±0.45	0.65±0.02	316.56±12.45
4m	5.76±0.54	0.67±0.02	306.34±10.32
Resveratrol	7.43±0.50	0.32±0.02	294.22±10.23
Control	5.39±0.23	0.26±0.01	216.12±11.34

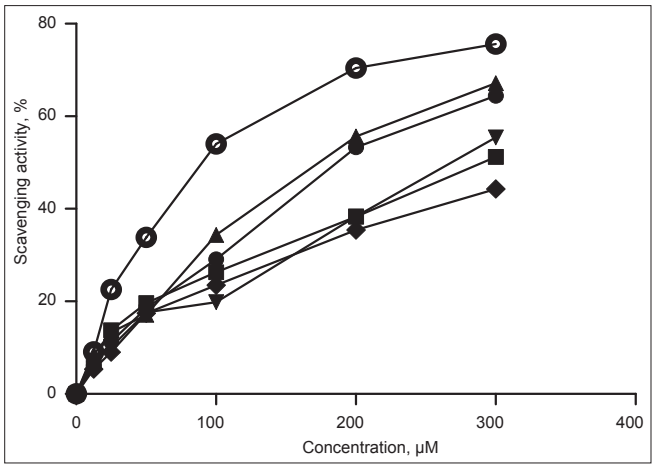

Fig. 2: Scavenging activity of compound 4(a-e) on DPPH radical. -●- 4-NO_2; -■-3-NO_2; -▲- 4-F; -▼- 4-Cl; -♦-4-Br; -O- Resveratrol (Control)

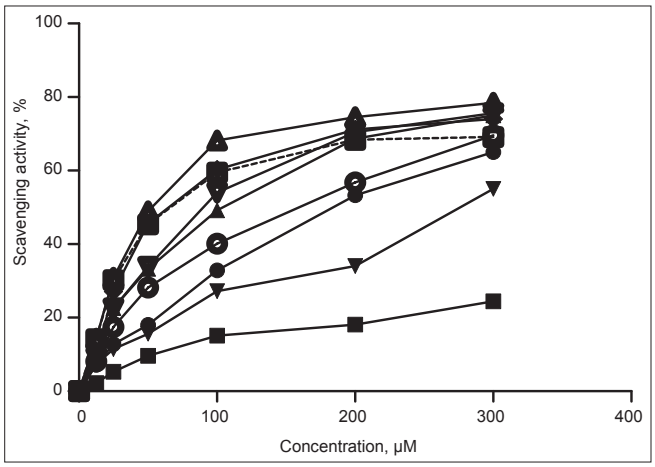

Fig. 3: Scavenging activity of compound 4(f-m) on DPPH radical. -●- 4-Me; -■- 4-iPr; -▲- 4-OH; -▼- 3-OMe; -♦- 3-Ome-4-OH; -O- 3-OH-4-OMe; -□- 3-OEt-4-OH; -Δ- 4-NMe_2; -∇- Resveratrol (Control)

elevation of free radical scavenging enzyme activities such as SOD, catalase and GSH-px. As shown in Table 1, some of these compounds (4k, 4l and 4m) caused significant elevation of SOD activity. Similar results were obtained in case of the catalase and the GSH-px activities as shown in Table 3.

In conclusion, a series of substituted benzaldehyde (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl) thiosemicarbazones have been synthesised from 2,3,4,6-tetra-*O*-acetyl-β-D-galctopyranosyl 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.

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