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# Bioorganic & Medicinal Chemistry Letters

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## Syntheses and evaluation of glucosyl aryl thiosemicarbazide and glucosyl thiosemicarbazone derivatives as antioxidant and anti-dyslipidemic agents<sup>☆</sup>

Samir Ghosh<sup>a,b</sup>, Anup Kumar Misra<sup>a,b,\*</sup>, Gitika Bhatia<sup>c</sup>, M. M. Khan<sup>c</sup>, A. K. Khanna<sup>c</sup><sup>a</sup> Division of Molecular Medicine, Bose Institute, P-1/12, C.I.T. Scheme VII-M, Kolkata 700054, India<sup>b</sup> Medicinal and Process Chemistry Division, Central Drug Research Institute, Lucknow 226001, India<sup>c</sup> Biochemistry Division, Central Drug Research Institute, Lucknow 226001, India

### ARTICLE INFO

#### Article history:

Received 18 August 2008

Revised 14 November 2008

Accepted 19 November 2008

Available online 24 November 2008

#### Keywords:

Thiosemicarbazide

Thiosemicarbazone

Antidyslipidemic

Antioxidant

Cholesterol

### ABSTRACT

A series of *N*-per-*O*-acetyl-glucosyl arylthiosemicarbazide and thiosemicarbazone derivatives have been synthesized and evaluated for their in vivo anti-dyslipidemic and in vitro antioxidant activities. Among 16 compounds tested, 3 compounds showed potent anti-dyslipidemic activity and 6 compounds showed potent antioxidant and scavenger of oxygen free radicals activity.

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Atherosclerosis and its associated complications is now the major cause of myocardial morbidity and mortality worldwide. Elevated level of plasma concentration of cholesterol, especially low density lipoprotein (LDL) and triglyceride along with free radical oxidative stress are recognized as leading cause in the development of atherosclerosis and coronary heart disease.<sup>2</sup> In general, oxidative damage takes place in the Low density lipoprotein (LDL) of plasma by the hydroxyl radicals ( $\cdot\text{OH}$ ) generated by the metal ions present in the serum due to the alterations in their oxidation states. It has been demonstrated that oxidative damaged LDL are relatively more atherogenic than the native LDL.<sup>3</sup> Currently, several drugs are being used in the treatment of dyslipidemia.<sup>4</sup> The drugs can intervene by lowering cholesterol (LDL and total cholesterol) or by lowering triglyceride levels in plasma. Treatment of hyperlipidemia using statins has been used to lower serum levels of cholesterol and triglyceride besides their known side effects such as, myositis, arthralgias, gastrointestinal upset and elevated liver function tests. Statins such as atorvastatin, lovastatin, fluvastatin, simvastatin and pravastatin act as inhibitors of HMG CoA reductase, an enzyme involved in the de novo synthesis of cholesterol and upgradation of LDA receptors in livers. There-

fore, it is essential to develop therapeutics for the treatment of hyperlipidemia reducing their severe side effects.

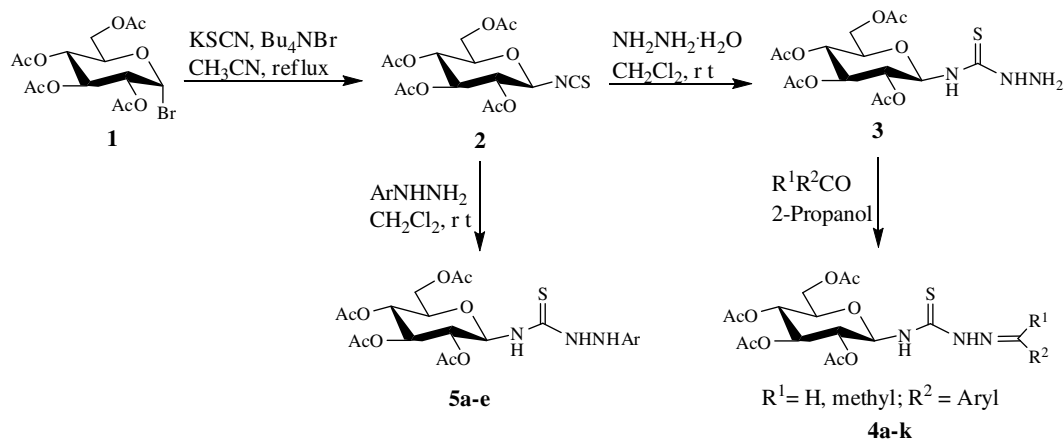
The involvement of hydroxyl free radicals ( $\cdot\text{OH}$ ) has been found to be a major causative factor for the peroxidative damage to lipoproteins present in the blood, which are responsible for the initiation and progression of atherosclerosis in the hyperlipidemic subject.<sup>5</sup> Hyperlipidemia may also induce other abnormalities like oxidation of free fatty acids, leading to the formation of ketone bodies as well as masking liver and muscles resistance to insulin which initiates the progress of diabetes in patients.<sup>6</sup> Furthermore, in hyperglycemic patients, several non-enzymatic glycosylation occurs accompanied by glucose oxidation catalyzed by  $\text{Cu}^{2+}$  and  $\text{Fe}^{2+}$  resulting in the formation of  $\text{O}_2\cdot^-$  and  $\cdot\text{OH}$  radicals which further accelerates the risk of cardiac diseases in dyslipidemic patients.<sup>7</sup>

Therefore, it is envisaged that, beside a cholesterol lowering property, a hypolipidemic agent that incorporates antioxidant activity will be able to protect endothelial and myocardial function and could serve as a better anti-atherosclerotic agent. Recently, we noted few papers in which thiosemicarbazides and related compounds have been evaluated for the free radical scavenger activity.<sup>8</sup> Prompted by the reports, we envisaged that glucosyl thiosemicarbazides or thiosemicarbazone could be useful in controlling metabolic disorder such as dyslipidemia and scavenging of free radicals. In order improve the solubility of thiosemicarbazide derivatives we prepared a series of per-*O*-acetylglucosyl thiosemicarbazide and semicarbazone derivatives and evaluated them for anti-dyslipidemic activity in vivo and antioxidant activity in vitro.

<sup>☆</sup> See Ref. 1.

\* Corresponding author. Present address: Division of Molecular Medicine, Bose Institute, P-1/12, C.I.T. Scheme VII-M, Kolkata 700054, India. Tel.: +91 33 2569 3240; fax: +91 33 2355 3886.

E-mail address: [akmisra69@rediffmail.com](mailto:akmisra69@rediffmail.com) (A.K. Misra).



**Scheme 1.** Synthesis of glucosyl thiosemicarbazone (**4a–k**) and glucosyl aryl thiosemicarbazide derivatives (**5a–e**) from glucosyl isothiocyanate (**2**).

A number of glucosyl thiosemicarbazide derivatives showed significant *in vivo* anti-dyslipidemic and *in vitro* antioxidant activity, which could be used as leads for the development of effective anti-atherosclerotic agents. We report herein, the synthesis and anti-dyslipidemic and antioxidant activities of a series of glucosyl thiosemicarbazide derivatives.

Preparation of a series of per-O-acetylated glucosyl thiosemicarbazone (**4a–k**) and per-O-acetylated glucosyl aryl thiosemicarbazide derivatives (**5a–e**) is presented in Scheme 1. Per-O-acetylated  $\beta$ -D-glucosyl isothiocyanate derivative (**2**)<sup>9</sup> was prepared from commercially available acetobromo-D-glucose (**1**) using  $\text{KSCN}$  and  $\text{Bu}_4\text{NBr}$  under reflux for 4 h. A portion of compound **2** was treated with hydrazine monohydrate in  $\text{CH}_2\text{Cl}_2$  at room temperature to furnish compound **3** in 92% yield.<sup>10</sup> Compound **3** was allowed to react with a series of aldehydes and ketones using 5–10 drops of acetic acid as catalyst in 2-propanol at 80 °C to give compounds **4a–k** in excellent yield.<sup>11</sup> In another experiment, compound **2** was allowed to react with a series of aryl hydrazines in  $\text{CH}_2\text{Cl}_2$  to furnish compounds **5a–e** in excellent yield.<sup>12</sup> The purity of these compounds was checked by TLC and spectral analysis (Table 1 and 2).

The anti-dyslipidemic activities of compounds **4a–k** and **5a–e** were evaluated in a *in vivo* Triton model.<sup>13–15</sup> Administration of triton WR-1339 in rats induced marked hyperlipidemia as evidenced by increase in the plasma levels of total cholesterol (TC) (3.53 F), phospholipids (PL) (3.06 F), triglyceride (Tg) (3.13 F). Triton induced rats caused inhibition of post heparin lipolytic activity

(PHLA)<sup>16</sup> (–33%) as compared to control (Table 3). Treatment of hyperlipidemic rats with compounds **4a–k** and **5a–e** at dose of 100 mg/Kg po reversed the plasma level of lipids with varying extents.<sup>17</sup> The effect of compounds **4a**, **4d** and **5a** showed potent lipid lowering activity in plasma level of TC, PL and Tg by 22%, 24%, 20%; 26%, 25%, 19% and 26%, 20%, 19%, respectively, while other compounds showed mild lipid lowering activity as compared to triton. These data were compared with Gemfibrozil at a dose of 100 mg/Kg, which showed a decrease in plasma levels of TC, PL and Tg by 38%, 39% and 37%, respectively. In PHLA, the treatment with compound partially reactivated these lipolytic activities in plasma of hyperlipidemic rats. However, gemfibrozil causes the significant reversal of these enzymes level.

**Table 2**  
synthesis of per-O-acetylated glucosyl arylthiosemicarbazide derivatives (**5a–e**)

| Entry | Compounds ( <b>5a–e</b> )           | Yield (%) | mp (°C) |
|-------|-------------------------------------|-----------|---------|
|       |                                     |           |         |
| 1     | <b>5a</b> : Ar = 2,4-difluorophenyl | 84        | Oil     |
| 2     | <b>5b</b> : Ar = 4-fluorophenyl     | 85        | 135–37  |
| 3     | <b>5c</b> : Ar = 4-methoxyphenyl    | 82        | Oil     |
| 4     | <b>5d</b> : Ar = phenyl             | 90        | Oil     |
| 5     | <b>5e</b> : Ar = 2,4-dinitrophenyl  | 88        | Oil     |

**Table 1**  
Synthesis of per-O-acetylated glucosyl arylthiosemicarbazone derivatives (**4a–k**)

| Entry | Compounds ( <b>4a–k</b> )                                                            | Time (h) | Yield (%) | mp (°C) |
|-------|--------------------------------------------------------------------------------------|----------|-----------|---------|
|       |                                                                                      |          |           |         |
| 1     | <b>4a</b> : $\text{R}^1 = \text{methyl}$ , $\text{R}^2 = 4\text{-bromophenyl}$       | 4        | 92        | 186–88  |
| 2     | <b>4b</b> : $\text{R}^1 = \text{methyl}$ , $\text{R}^2 = 3,4\text{-dimethoxyphenyl}$ | 5        | 90        | 200–201 |
| 3     | <b>4c</b> : $\text{R}^1 = \text{methyl}$ , $\text{R}^2 = 3\text{-aminophenyl}$       | 4        | 94        | 178–80  |
| 4     | <b>4d</b> : $\text{R}^1 = \text{methyl}$ , $\text{R}^2 = 3\text{-nitrophenyl}$       | 4        | 92        | 184–86  |
| 5     | <b>4e</b> : $\text{R}^1 = \text{methyl}$ , $\text{R}^2 = 2,5\text{-dimethylphenyl}$  | 5        | 88        | Oil     |
| 6     | <b>4f</b> : $\text{R}^1 = \text{H}$ , $\text{R}^2 = 4\text{-chlorophenyl}$           | 1.5      | 97        | 198–200 |
| 7     | <b>4g</b> : $\text{R}^1 = \text{H}$ , $\text{R}^2 = 4\text{-methoxyphenyl}$          | 4        | 94        | 182–83  |
| 8     | <b>4h</b> : $\text{R}^1 = \text{H}$ , $\text{R}^2 = 5\text{-nitrothiophen-2-yl}$     | 3        | 85        | 178–80  |
| 9     | <b>4i</b> : $\text{R}^1 = \text{H}$ , $\text{R}^2 = 4\text{-nitrophenyl}$            | 2        | 96        | 200–03  |
| 10    | <b>4j</b> : $\text{R}^1 = \text{H}$ , $\text{R}^2 = 4\text{-pyridyl}$                | 2        | 90        | 102–103 |
| 11    | <b>4k</b> : $\text{R}^1 = \text{H}$ , $\text{R}^2 = 3\text{-pyridyl}$                | 2        | 95        | 134–36  |

**Table 3**Lipid lowering and post heparin lipolytic activity of compounds **4a–k** and **5a–e** in triton treated hyperlipidemic rats

| Entry | Test compounds       | TC <sup>a</sup>             | PL <sup>a</sup>            | Tg <sup>a</sup>             | Protein <sup>b</sup>      | PHLA <sup>c</sup>     |
|-------|----------------------|-----------------------------|----------------------------|-----------------------------|---------------------------|-----------------------|
| 1     | Control              | 90.65 ± 1.28                | 79.05 ± 4.96               | 86.31 ± 11.21               | 6.23 ± 0.43               | 16.66 ± 1.14          |
| 2     | Triton only          | 320.41*** ± 11.27 (+3.53 F) | 245.13*** ± 4.86 (+3.06 F) | 270.83*** ± 14.43 (+3.13 F) | 12.99*** ± 0.33 (+2.08 F) | 11.16*** ± 1.37 (–33) |
| 3     | Triton + <b>4a</b>   | 250.62*** ± 4.96 (–22)      | 180.55*** ± 8.33 (–26)     | 202.62*** ± 3.24 (–25)      | 9.60*** ± 0.42 (–26)      | 13.36*** ± 2.22 (+16) |
| 4     | Triton + <b>4b</b>   | 260.41** ± 22.17 (–19)      | 192.58*** ± 4.24 (–23)     | 211.79*** ± 5.22 (–22)      | 9.75*** ± 0.66 (–24)      | 14.88** ± 1.88 (+25)  |
| 5     | Triton + <b>4c</b>   | 270.00** ± 6.50 (–16)       | 208.46* ± 4.37 (–15)       | 243.50* ± 3.68 (–13)        | 10.33** ± 0.33 (–20)      | 12.26* ± 1.14 (+9)    |
| 6     | Triton + <b>4d</b>   | 245.83*** ± 10.97 (–23)     | 197.68** ± 3.49 (–19)      | 221.85** ± 8.81 (–18)       | 10.49** ± 0.33 (–19)      | 12.41* ± 1.16 (+10)   |
| 7     | Triton + <b>4e</b>   | 264.58** ± 13.01 (–18)      | 201.38** ± 6.94 (–18)      | 222.50** ± 9.01 (–17)       | 11.44* ± 0.53 (–12)       | 12.41* ± 0.90 (+10)   |
| 8     | Triton + <b>4f</b>   | 274.68* ± 17.06 (–14)       | 229.16NS ± 6.94 (–6)       | 243.87* ± 16.86 (–10)       | 10.02*** ± 0.35 (–22)     | 12.77* ± 0.88 (+12)   |
| 9     | Triton + <b>4g</b>   | 265.00** ± 17.21 (–17)      | 207.82* ± 7.93 (–15)       | 235.83* ± 5.20 (–10)        | 10.93* ± 0.68 (–15)       | 12.17NS ± 0.96 (+8)   |
| 10    | Triton + <b>4h</b>   | 276.35* ± 9.96 (–13)        | 205.60** ± 5.62 (–16)      | 230.58* ± 4.41 (–14)        | 10.40** ± 0.85 (–19)      | 13.0* ± 1.02 (+14)    |
| 11    | Triton + <b>4i</b>   | 260.24* ± 8.89 (–19)        | 209.88** ± 6.32 (–14)      | 240.88* ± 9.35 (–11)        | 10.14* ± 0.55 (–22)       | 12.67* ± 0.86 (+13)   |
| 12    | Triton + <b>4j</b>   | 286.44** ± 9.97 (–11)       | 217.76* ± 7.88 (–11)       | 248.32* ± 7.84 (–8)         | 11.52** ± 0.76 (–11)      | 12.10NS ± 3.46 (+8)   |
| 13    | Triton + <b>4k</b>   | 270.77** ± 11.24 (–15)      | 210.88* ± 9.96 (–14)       | 242.66* ± 7.44 (–10)        | 11.12* ± 0.45 (–14)       | 12.22NS ± 0.86 (+9)   |
| 14    | Triton + <b>5a</b>   | 242.70*** ± 13.01 (–24)     | 186.10*** ± 5.00 (–25)     | 202.08*** ± 9.54 (–25)      | 10.33** ± 1.04 (–20)      | 14.64*** ± 2.08 (+23) |
| 15    | Triton + <b>5b</b>   | 280.20* ± 13.00 (–13)       | 209.71* ± 11.02 (–14)      | 245.00* ± 6.61 (–9)         | 10.61** ± 0.78 (–18)      | 12.99* ± 0.96 (+14)   |
| 16    | Triton + <b>5c</b>   | 265.10** ± 14.18 (–18)      | 215.83* ± 7.01 (–12)       | 243.75* ± 2.50 (–10)        | 10.62** ± 0.10 (–18)      | 12.08NS ± 2.90 (+7)   |
| 17    | Triton + <b>5d</b>   | 283.33* ± 18.30 (–11)       | 222.40* ± 2.77 (–10)       | 245.50* ± 18.87 (–9)        | 10.88** ± 0.50 (–16)      | 12.17* ± 1.72 (+8)    |
| 18    | Triton + <b>5e</b>   | 278.78** ± 16.22 (–13)      | 220.46* ± 6.78 (–10)       | 247.88* ± 17.27 (–8)        | 11.23* ± 0.92 (–13)       | 13.12* ± 2.10 (+17)   |
| 19    | Triton + gemfibrozil | 200.22*** ± 17.11 (–38)     | 152.11*** ± 11.11 (–39)    | 170.33*** ± 12.23 (–37)     | 8.60*** ± 0.27 (–33)      | 16.93*** ± 1.00 (+34) |

TC, total cholesterol; PL, phospholipid; Tg, triglyceride; PHLA, post-heparin lipolytic activity.

Values are mean ± SD of six animals; \**P* < 0.01; \*\**P* < 0.05; \*\*\**P* < 0.001; NS, not significant.

Triton treated group compared with control and Triton plus compound treated group.

<sup>a</sup> mg/dL.<sup>b</sup> g/dL.<sup>c</sup> nmol free fatty acid formed/h/mL plasma.**Table 4**Effect of compounds **4a–k** and **5a–e** on the generation of superoxide and hydroxyl radical and lipid peroxidation in microsomes

| Test compound | Conc. of compd. (μg/mL) | Generation of superoxide ions (O <sub>2</sub> <sup>•−</sup> ) <sup>a</sup> | Generation of hydroxyl radicals (•OH) <sup>b</sup> | Microsomal lipid peroxidation <sup>b</sup>  |
|---------------|-------------------------|----------------------------------------------------------------------------|----------------------------------------------------|---------------------------------------------|
| Control       |                         | 90.38 ± 7.12                                                               | 75.52 ± 5.87                                       | 88.37 ± 9.14                                |
| <b>4a</b>     | 100 200                 | 70.97 ± 4.92** (–21) 60.45 ± 3.84*** (–33)                                 | 65.11 ± 4.92* (–14) 57.30 ± 5.0*** (–24)           | 74.05 ± 5.33** (–16) 62.46 ± 2.84*** (–29)  |
| <b>4b</b>     | 100 200                 | 82.51 ± 4.77NS (–9) 67.95 ± 5.3*** (–24)                                   | 64.37 ± 3.88* (–15) 55.15 ± 4.87*** (–27)          | 68.56 ± 7.39** (–22) 56.67 ± 5.0*** (–35)   |
| <b>4c</b>     | 100 200                 | 69.51 ± 4.71** (–23) 55.97 ± 3.11*** (–38)                                 | 60.27 ± 7.12** (–20) 46.70 ± 4.11*** (–38)         | 71.78 ± 5.71** (–19) 57.15 ± 2.84*** (–35)  |
| <b>4d</b>     | 100 200                 | 75.08 ± 5.31 (–17) 60.21 ± 5.60 (–33)                                      | 57.47 ± 4.32*** (–24) 48.86 ± 5.00*** (–35)        | 70.48 ± 5.30** (–20) 61.53 ± 5.0*** (–30)   |
| <b>4e</b>     | 100 200                 | 72.11 ± 5.62** (–20) 63.22 ± 4.12*** (–30)                                 | 62.77 ± 4.66* (–16) 53.33 ± 3.87*** (–29)          | 73.88 ± 4.88** (–16) 65.32 ± 5.32*** (–26)  |
| <b>4f</b>     | 100 200                 | 75.69 ± 5.39* (–16) 67.25 ± 4.42*** (–25)                                  | 65.35 ± 5.22* (–13) 61.02 ± 4.33** (–19)           | 67.26 ± 3.72** (–23) 58.35 ± 2.88*** (–33)  |
| <b>4g</b>     | 100 200                 | 67.00 ± 4.37*** (–25) 60.20 ± 4.88*** (–33)                                | 60.75 ± 5.18*** (–20) 49.73 ± 4.44*** (–34)        | 64.96 ± 3.81*** (–26) 57.32 ± 2.88*** (–35) |
| <b>4h</b>     | 100 200                 | 72.19 ± 5.33** (–20) 59.03 ± 4.44*** (–34)                                 | 60.65 ± 5.21** (–20) 47.50 ± 3.11*** (–37)         | 78.31 ± 5.37* (–11) 67.20 ± 3.00*** (–23)   |
| <b>4i</b>     | 100 200                 | 78.30 ± 5.66*** (–13) 69.78 ± 3.94* (–23)                                  | 62.90 ± 5.22** (–17) 58.23 ± 3.33** (–23)          | 72.45 ± 3.65** (–18) 60.67 ± 2.98** (–31)   |
| <b>4j</b>     | 100 200                 | 77.68 ± 5.26** (–14) 67.86 ± 4.22* (–25)                                   | 67.45 ± 5.66* (–11) 56.78 ± 3.90*** (–23)          | 68.87 ± 5.50* (–22) 58.98 ± 3.84*** (–33)   |
| <b>4k</b>     | 100 200                 | 75.78 ± 4.44* (–16) 64.88 ± 3.83** (–28)                                   | 65.88 ± 3.45*** (–13) 55.43 ± 3.21* (–26)          | 72.86 ± 5.0** (–18) 65.77 ± 3.12*** (–26)   |
| <b>5a</b>     | 100 200                 | 75.20 ± 5.88* (–16) 66.06 ± 4.44*** (–27)                                  | 59.90 ± 5.01** (–20) 48.44 ± 3.77*** (–35)         | 69.76 ± 3.00*** (–24) 59.50 ± 2.77*** (–31) |
| <b>5b</b>     | 100 200                 | 76.18 ± 5.33* (–15) 63.90 ± 3.92*** (–29)                                  | 58.59 ± 2.87*** (–22) 48.41 ± 3.99*** (–36)        | 68.69 ± 6.44** (–22) 57.60 ± 3.64*** (–34)  |
| <b>5c</b>     | 100 200                 | 71.04 ± 6.0** (–21) 56.65 ± 3.70*** (–37)                                  | 64.12 ± 5.70* (–15) 57.81 ± 4.23*** (–23)          | 63.45 ± 5.11*** (–28) 59.99 ± 3.77*** (–32) |
| <b>5d</b>     | 100 200                 | 72.43 ± 5.77** (–19) 61.29 ± 5.22*** (–32)                                 | 56.24 ± 3.91*** (–25) 43.87 ± 2.86*** (–42)        | 67.51 ± 3.98*** (–23) 53.88 ± 2.11*** (–39) |
| <b>5f</b>     | 100 200                 | 70.56 ± 5.68** (–22) 64.89 ± 4.0*** (–28)                                  | 61.78 ± 4.12** (–18) 55.45 ± 2.67** (–26)          | 68.23 ± 3.56*** (–23) 56.88 ± 3.56*** (–36) |
| Standard drug | 200                     | 20.50 ± 0.04*** (–77) Allopurinol                                          | 41.30 ± 1.73*** (–45) Mannitol                     | 41.83 ± 0.03*** (–53) α-Tocopherol          |

Each value is mean ± SD of six rats. \*\*\**P* < 0.001; \*\**P* < 0.05; \**P* < 0.01; NS, not significant. Experimental data compared with control experiment.<sup>a</sup> nmol formazone formed/min.<sup>b</sup> nmol MDA formed/h/mg protein.

In another experiment, antioxidant activities of compounds **4a–k** and **5a–e** were evaluated by generating free radicals<sup>18</sup> in vitro in the absence and presence of these compounds. The scavenging potential of compounds **4a–k** and **5a–e** at 100 and 200 μg/mL against formation of O<sub>2</sub><sup>•−</sup> are presented in Table 2. Compounds **4d**, **4h** and **5a** caused significant decrease in plasma levels of lipid in triton model of Hyperlipidemia. Triton WR-1339 acts as surfactant, suppresses the action of lipase and blocks the uptake of lipoproteins from the circulation of extra hepatic tissues resulted an increase in the levels of circulatory lipids.<sup>19</sup> Compounds **4a**, **4c**, **4d**, **4g**, **4h** and **5c** showed significant antioxidant and scavenger of oxygen free radicals possibly through metal ion chelation and xanthine

oxidase inhibition in an in vitro model of non-enzymatic and enzymatic lipid peroxidation<sup>20</sup> (see Table 4).

In conclusion a series of novel glucosyl aryl thiosemicarbazide and glucosyl thiosemicarbazone derivatives have been synthesized and shown to be effective anti-dyslipidemic and antioxidant agents. Further optimization of the lead molecules are currently in progress in our laboratory.

## Acknowledgments

Instrumentation facilities from SAIF, CDRI are gratefully acknowledged. S.G. thanks CSIR, New Delhi, for providing a Junior

Research Fellowship. A.K.M. thanks DST, New Delhi for Ramanna fellowship.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.bmcl.2008.11.070](https://doi.org/10.1016/j.bmcl.2008.11.070).

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- Preparation of N-(2,3,4,6-tetra-O-acetyl- $\beta$ -D-glucopyranosyl) thiosemicarbazide (3):** To a solution of compound **2** (5 g, 12.85 mmol) in  $\text{CH}_2\text{Cl}_2$  (100 mL) was added hydrazine monohydrate (1.2 g, 24 mmol) and the reaction mixture was allowed to stir at room temperature for 2 h. The solvents were removed under reduced pressure and the crude product was purified over  $\text{SiO}_2$  using hexane-EtOAc (2:1) as eluant to furnish pure compound **3** (5.1 g, 94%).
- Typical experimental condition for the preparation of compounds (4a–k):** To a solution of compound **3** (500 mg, 1.2 mmol) in 2-propanol (5 mL) was added appropriate aldehyde or ketone (1.4 mmol) followed by 5 drops of AcOH and the reaction mixture was allowed to stir at 80 °C for appropriate time (Table 1). The solvents were removed under reduced pressure and the crude product was purified over  $\text{SiO}_2$  using hexane-EtOAc (2:1) as eluant to furnish pure compound **4a–k**.
- Typical experimental condition for the preparation of compounds (5a–e):** To a solution of compound **2** (500 mg, 1.28 mmol) in anhydrous  $\text{CH}_2\text{Cl}_2$  (5 mL) was added appropriate aryl hydrazine (1.1 mmol) and the reaction mixture was allowed to stir at room temperature for 2 h. The solvents were removed under reduced pressure and the crude product was purified over  $\text{SiO}_2$  using hexane-EtOAc (2:1) as eluant to furnish pure compound **5a–e**.
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- Lipid lowering activity:** Adult male Charles Foster rats ( $200 \pm 225$  g) bred in the animal house of the institute were used for the lipid lowering activity. Rats were divided in control, triton induced, triton plus compounds and Gemfibrozil (100 mg/Kg) treated groups containing six rats in each. Hyperlipidemia was developed by administration of Triton WR-1339 (Sigma chemical co., St. Louis, USA) at a dose of 400 mg/Kg body wt. intraperitoneally to animals of all groups except the control. Compounds **4a–k** and **5a–e** were macerated with gum acacia (0.2% w/v), suspended in water and fed simultaneously with triton at a dose of 100 mg/Kg po to the animals of treated groups. Animals of the control and triton group without treatment with test compounds were given same amount of gum acacia suspension (vehicle). After 18 h of treatment (50 mg/kg b. wt.) 1.0 mL blood was withdrawn from retro-orbital sinus using glass capillary in EDTA coated eppendorf tube (3.0 mg/mL blood). The blood was centrifuged (at 2500 g) at 4 °C for 10 min and the plasma was separated. Plasma was diluted with normal saline (ratio 1:3) and used for analysis of total cholesterol (TC), phospholipids (PL), triglycerides (Tg) by standard procedures.
- Antioxidant activity (generation of free radicals):** Super oxide anions ( $\text{O}_2^{\cdot-}$ ) were generated enzymatically by xanthine (160 mM), xanthine oxidase (0.04 U), and nitroblue tetrazolium (320  $\mu\text{M}$ ) in absence or presence of compounds **4a–k** and **5a–e** (100  $\mu\text{g/mL}$ ) in 100 mM phosphate buffer (pH 8.2). Fractions were sonicated well in phosphate buffer before use. The reaction mixtures were incubated at 37 °C and after 30 min the reaction was stopped by adding 0.5 mL glacial acetic acid. The amount of formazone formed was calculated spectrophotometrically. In another set of experiment effect of compounds on the generation of hydroxyl radical ( $\text{OH}^{\cdot}$ ) was also studied by non-enzymatic reactants. Briefly,  $\text{OH}^{\cdot}$  were generated in a non-enzymatic system comprising deoxy ribose (2.8 mM),  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  (2 mM), sodium ascorbate (2.0 mM) and  $\text{H}_2\text{O}_2$  (2.8 mM) in 50 mM  $\text{KH}_2\text{PO}_4$  buffer (pH 7.4) to a final volume of 2.5 mL. The above reaction mixtures in the absence or presence of test compounds (100  $\mu\text{g/mL}$  and 200  $\mu\text{g/mL}$ ) were incubated at 37 °C for 90 min. The test compounds were also studied for their inhibitory action against microsomal lipid peroxidation in vitro by non-enzymatic inducer. Reference tubes and reagents blanks were also run simultaneously. Malondialdehyde (MDA) contents in both experimental and reference tubes were estimated spectrophotometrically by thiobarbituric acid as mentioned above. Allopurinol, Mannitol and  $\alpha$ -tocopherol were used as standard drugs for superoxide, hydroxylations and microsomal lipid peroxidation. All experimental data were analyzed using Student's *t*-test. Oxidized LDL was compared with the test compounds treated oxidized LDL. The generation of oxygen free radicals were compared in the presence and absence of test compounds. The hyperlipidemic group was compared with control and hyperlipidemic plus drug treated groups  $P < 0.05$  was considered to be significant.
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