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# Novel carbocyclic nucleoside analogs suppress glomerular mesangial cells proliferation and matrix protein accumulation through ROS-dependent mechanism in the diabetic milieu. II. Acylhydrazone-functionalized pyrimidines

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ABSTRACT

We report herein the synthesis of a novel series of carbocyclic acylhydrazone derivatives of uracil, thymine and cytosine from the corresponding nucleic bases and their biological activity to treat diabetic nephropathy. Intriguingly, five derivatives significantly reduced high-glucose induced glomerular mesangial cells proliferation and matrix protein accumulation in vitro. The anti-oxidative effects displayed by these molecules suggest that their activity might involve a ROS-dependent mechanism.



Diabetes mellitus is a group of metabolic disorders characterized by persistent hyperglycemia either due to low secretion of insulin or cell resistance to it. It results in several complications, some of which are macro-vascular and others that are microvascular such as Diabetic Nephropathy (DN).

Diabetic Nephropathy is a progressive kidney disease caused by angiopathy of the capillaries in the kidney glomeruli. It is characterized by a progressive loss of the glomerular filtration rate and excessive deposition of extracellular matrix leading to end-stage renal disease that renders it the leading cause of premature death in young diabetics.[1–4]

The glomerular mesangial cells (MCs) are modified smooth muscle cells that lie between the capillaries and represent 30–40% of the total glomerular cell population. They are involved in several functions including the regulation of blood flow by their contractile activity and secretion of extracellular matrix, prostaglandins, and cytokines.

Furthermore, MCs regulate blood flow in the capillaries and maintain the glomerular structure. Their paramount importance has been recently elucidated in the early stages of DN during which mesangial cells proliferate for 24–48 h preceding a cell cycle arrest at the G zero (G0) phase followed by hypertrophy and extracellular matrix expansion.[5] This has been confirmed in cultured mesangial cells and animal models where hyperglycemia or high glucose treatment induced MC proliferation as well as matrix expansion.[6–8] This process, however, has been linked to the high glucose-induced generation of reactive oxygen species via numerous pathways such as stimulated glycolysis, tricarboxylic acid cycle, and 12-lipoxygenase pathway of arachidonic acid metabolism.[9–14]

Diabetes is a chronic disease, that may result in severe complications despite strict glycemic control and insulin treatment. This fact urged the need for new intervention strategies that target the disease as well as its complications from early onset. Acylhydrazone derivatives possess, among others, antimicrobial, anticonvulsant, analgesic, antiinflammatory, antiplatelet, antitubercular, and antitumoral activities.[15,16] Acylhydrazones of

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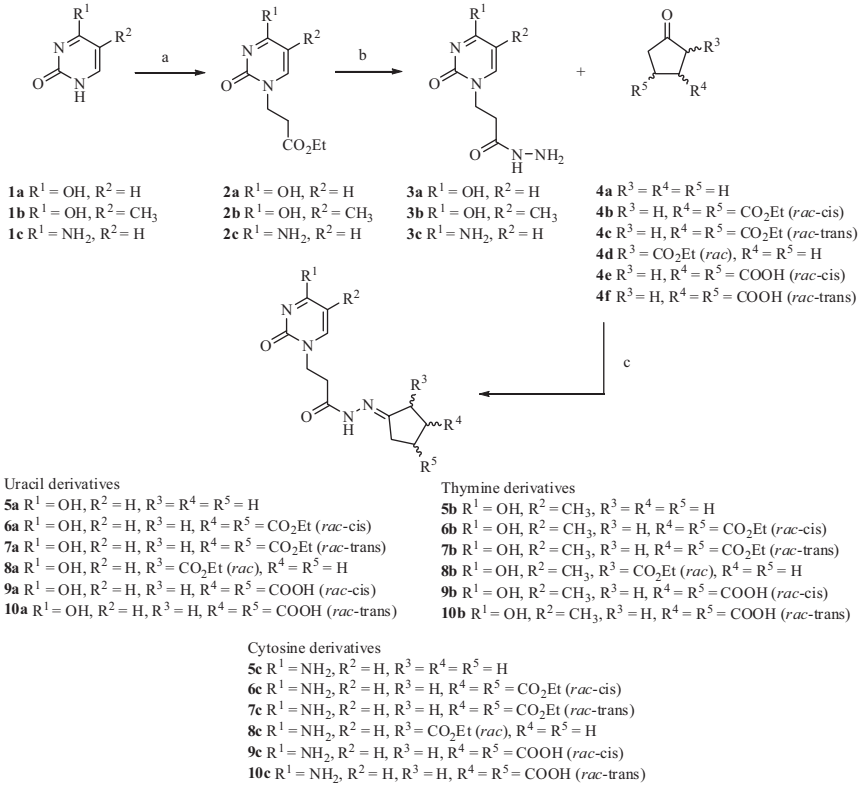


**Scheme 1.** Synthetic route of compounds **5a–10a**, **5b–10b** and **5c–10c**. Reagents and conditions: (a) ethyl acrylate, Na metal, EtOH, reflux, 24 h; (b) $N_2H_4{\cdot}H_2O$, EtOH, reflux, 24 h; (c) EtOH or MeOH, TFA, 90 °C in sealed tube, 1–3 h.

**Table 1**
Effect of hydrazides and carbocyclic nucleoside analogs on high-glucose induced fibronectin and β-actin expression[a]

| Samples | Fibronectin/β-actin (arbitrary units)-0.5 μM | Fibronectin/β-actin (arbitrary units)-1 μM | Fibronectin/β-actin (arbitrary units)-5 μM |
|---|---|---|---|
| NG | 100 | 100 | 100 |
| HG | 118.8699 ± 10.93[b] | 118.8699 ± 10.93[b] | 118.8699 ± 10.93[b] |
| HG + **7b** | 94.23 ± 11.78 | 90.81 ± 4.62[c] | 87.64 ± 8.6 |
| HG + **6b** | 91.91 ± 11.85 | 97.91 ± 20.66 | 81.06 ± 3.27[c] |
| HG + **9b** | 80.11 ± 12.84[c] | 72 ± 33.59[c] | 70.72 ± 31.77 |
| HG + **10b** | 78.37 ± 8.07[c] | 64.58 ± 17.23[c] | 63.12 ± 20.58[c] |
| HG + **5b** | 117.54 ± 23.3 | 126.24 ± 55.65 | 126.52 ± 46.32 |
| HG + **5a** | 100.46 ± 30.23[c] | 84.77 ± 24.76[c] | 64.3 ± 12.94[c] |
| HG + **7a** | 145.08 ± 85.42 | 131.56 ± 66.96 | 94.05 ± 21.8[c] |
| HG + **3a** | 101.12 ± 37.74 | 80.52 ± 21.78[c] | 85.4 ± 20.91[c] |
| HG + **3b** | 89.24 ± 14.73[c] | 94.04 ± 5.47[c] | 101.74 ± 10.53 |
| HG + **3c** | 76.55 ± 3.91[c] | 81.6 ± 10.87[c] | 80.82 ± 16.23 |
| HG + **7c** | 98.25 ± 18.7 | 96.09 ± 21.23 | 80.58 ± 13.22[c] |
| HG + **6c** | 95.13 ± 50.14 | 91.05 ± 14.81[c] | 124.99 ± 27.34 |
| HG + **5c** | 217.9 ± 78.62[c] | 212.7 ± 139.47 | 274.92 ± 157.21 |
| HG + **8a** | 126.92 ± 31.33 | 125.7 ± 23.76 | 106.01 ± 3.27[c] |
| HG + **8c** | 111.38 ± 44.98 | 119.38 ± 22.21[c] | 102.11 ± 11.41[c] |
| HG + **8b** | 88.82 ± 25.74[c] | 96.49 ± 40.16 | 91.58 ± 51.81 |
| HG + **6a** | 97.45 ± 16.29 | 93.72 ± 16.66 | 76.45 ± 12.5[c] |

[a] Lysates were prepared from rat glomerular mesangial cells serum-deprived for 24 h, then treated for 48 h with high-glucose (25 mM) in the presence or absence of 0.5 μM (2nd column in the table), 1 μM (3rd column in the table), or 5 μM (4th column in the table) in the presence or absence of the hydrazide or the corresponding nucleoside derivative.
[b] $P$ <0.05 versus NG (No Glucose).
[c] $P$ <0.05 versus HG (High Glucose).

isoniazid (INZ) have shown inhibitory activity in mice infected with various strains of *Mycobacterium tuberculosis*.[17] They have also revealed less toxicity in these mice than INZ.[17,18] Buu-Hoi et al.[19] synthesized some acylhydrazone derivatives that are less toxic than hydrazides due to the blockage of $-NH_2$ group. These findings further support the growing importance of the preparation of acylhydrazones as leading medicinal compounds in the pharmaceutical industry.[19]

In a recent publication, Frederico et al.[20] reported the effect of four acylhydrazones on diabetes by determining their role in lowering serum glucose levels as well as insulin secretion. Their findings supported one acylhydrazone as a potentially useful candidate