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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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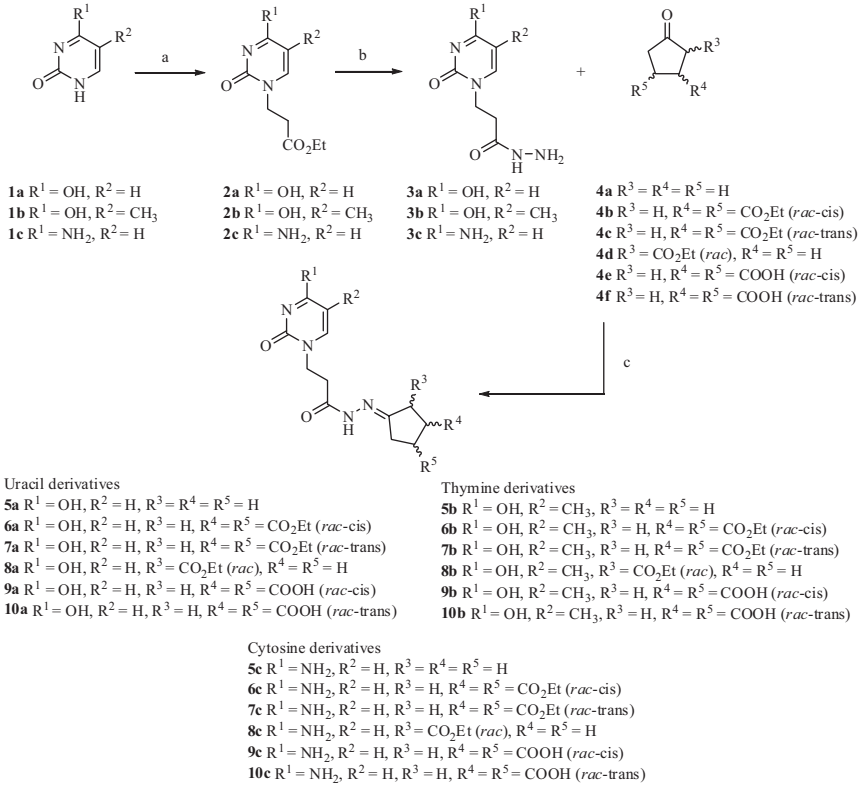

Scheme 1. Synthetic route of compounds **5a–10a**, **5b–10b** and **5c–10c**. Reagents and conditions: (a) ethyl acrylate, Na metal, EtOH, reflux, 24 h; (b) $N_2H_4{\cdot}H_2O$, EtOH, reflux, 24 h; (c) EtOH or MeOH, TFA, 90 °C in sealed tube, 1–3 h.

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

Table 2
Effect of cyclopentanones and carbocyclic nucleoside analogs on high-glucose induced fibronectin and β-actin expression[a]

Samples	Fibronectin/β-actin (arbitrary units) 0.25 μM	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	100
HG	119 ± 3.25[b]	119 ± 3.25[b]	119 ± 3.25[b]	119 ± 3.25[b]
HG + **4c**	82.4 ± 25.53	90.24 ± 14.28[c]	86.34 ± 33.26	79.74 ± 34.38
HG + **4b**	81.78 ± 31.7	79.83 ± 20.19[c]	88.94 ± 24.9	84.2 ± 17.26[c]
HG + **4a**	82.79 ± 8.56[c]	101.07 ± 19.45	113.72 ± 23.67	129.19 ± 47.6
HG + **4d**	109.35 ± 44.91	110.27 ± 49.03	88.3 ± 41.74[c]	137.12 ± 105.82
HG + **9a**	75.11 ± 8.16[c]	80.05 ± 15.79[c]	96.65 ± 39.1	116.73 ± 44.8
HG + **10a**	132.18 ± 58.37	115.58 ± 35.68	114.69 ± 32.53	96.94 ± 13.76[c]
HG + **9c**	135.58 ± 26.26[c]	143.09 ± 69.11	141.59 ± 92.7	112.87 ± 48.94
HG + **10c**	123.39 ± 10.84[c]	125.99 ± 45.41	128.39 ± 74.29	106.88 ± 78.94
HG + **4f**	135.08 ± 24.2[c]	143.35 ± 65.94	149.38 ± 60.35	124.24 ± 28.58
HG + **4e**	128.84 ± 20.28[c]	149.14 ± 79.13	144.68 ± 98.65	121.27 ± 62.6

[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 0.25 μM (2nd column in the table), 0.5 μM (3rd column in the table), 1 μM (4th column in the table), or 5 μM (5th column in the table) in the presence or absence of the cyclopentanone or the corresponding nucleoside derivative.
[b] P <0.05 versus NG.
[c] P <0.05 versus HG.

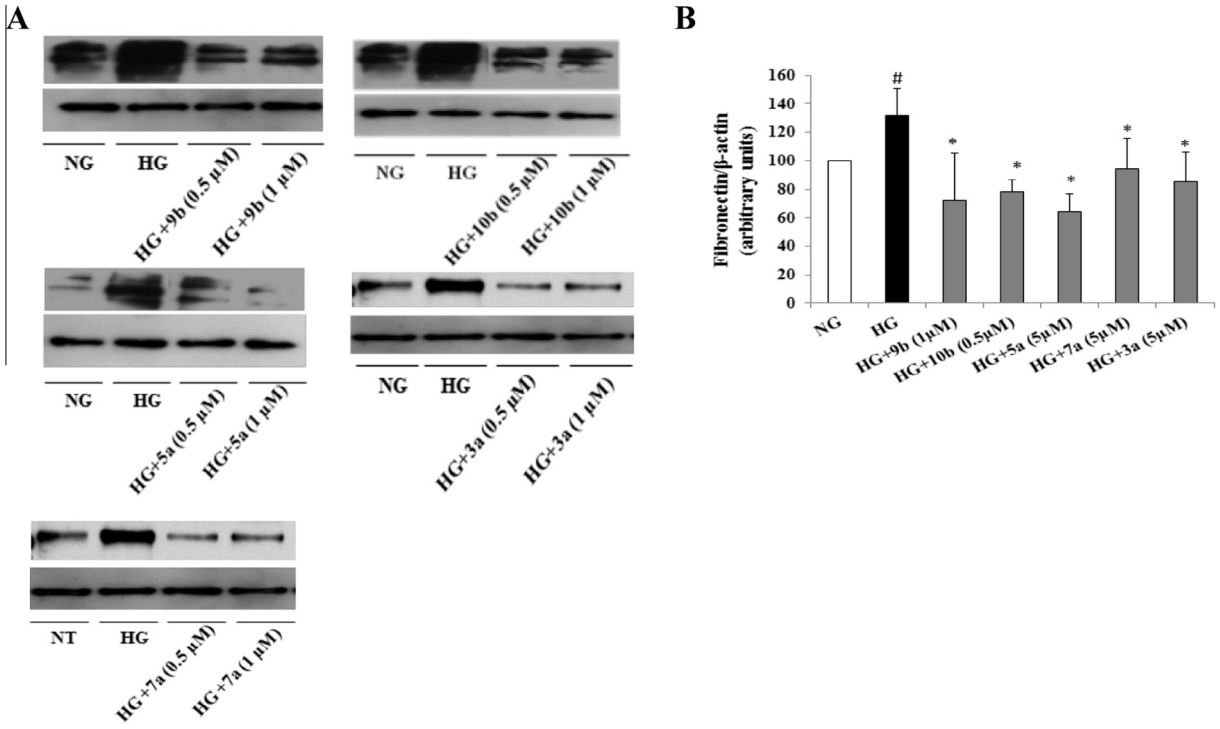

Figure 1. Effect of **9b**, **10b**, **5a**, **7a**, and **3a** on high-glucose induced fibronectin expression. (A) Depicts the representative Western blots of fibronectin (top), and β-actin (bottom) levels. (B) is the histogram showing quantitation of Western blot results from three different experiments. $^{\#}P$ <0.05 versus NG, $^{*}P$ <0.05 versus HG.

for diabetes therapy. Additionally, an increasing interest in carbocyclic nucleosides (carbanucleosides) has emerged in the recent years due to the wide range of biological activities they displayed as antitumor, antibiotic, antimicrobial, and antiviral agents.[21–26] More recently, we established the significant reduction in protein levels on high-glucose (HG)-induced mesangial cells proliferation induced by a novel series of carbocyclic nucleoside analogs.[27] We decided to investigate the combinatorial effect of both groups (nucleic base and acylhydrazone) in a series of pyrimidyl acylhydrazone derivatives (Scheme 1). We report herein the synthesis of a new series of carbocyclic derivatives of uridyl, thymidyl, and cytidyl acylhydrazones **5a**–**10a**, **5b**–**10b** and **5c**–**10c** (Scheme 1). We assessed their biological effect on (HG)-induced mesangial cells proliferation and (HG)-induced fibronectin expression. In addition, we investigated whether the reactive oxygen species (ROS)-dependent mechanism is associated with the reduction of protein expressions in vitro. The synthetic protocol followed in the preparation of the uridyl, thymidyl and cytidyl acylhydrazone derivatives **5a**–**10a**, **5b**–**10b** and **5c**–**10c** (Scheme 1) was initiated with the corresponding hydrazides **3a**–**3c** which were prepared following reported procedures.[28] In short, uracil, thymine or cytosine was allowed to react with ethyl acrylate in ethanol and catalytic amount of sodium metal following the Michael addition reaction to afford the corresponding ethyl esters **2a**–**2c**[29] in good yields. Hydrazinolysis of the ethyl ester group was carried out in ethanol under reflux, to yield the corresponding hydrazides **3a**–**3c**[28] which were condensed with equimolar amounts of cyclopentanone **4a** or a range of substituted cyclopentanones

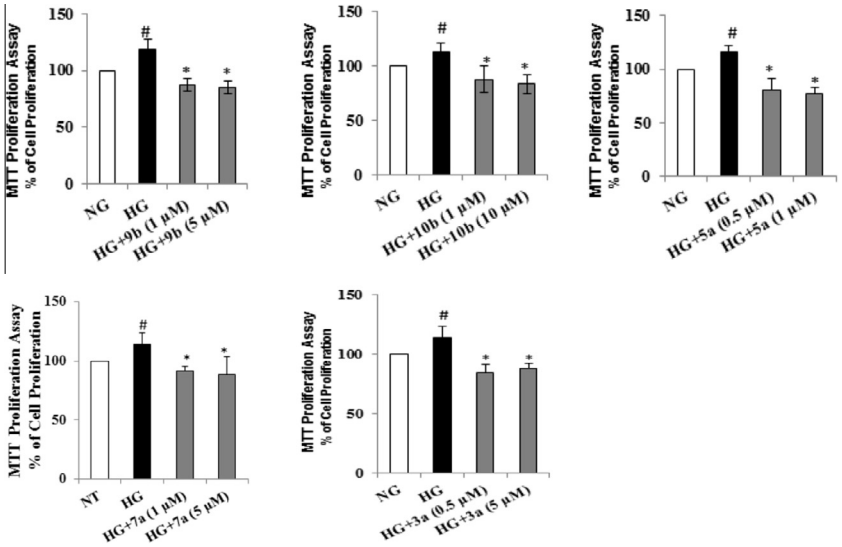

Figure 2. In vitro reduction of high-glucose induced glomerular mesangial cells' proliferation. $^{\#}P < 0.05$ versus NG, $^{*}P < 0.05$ versus HG.

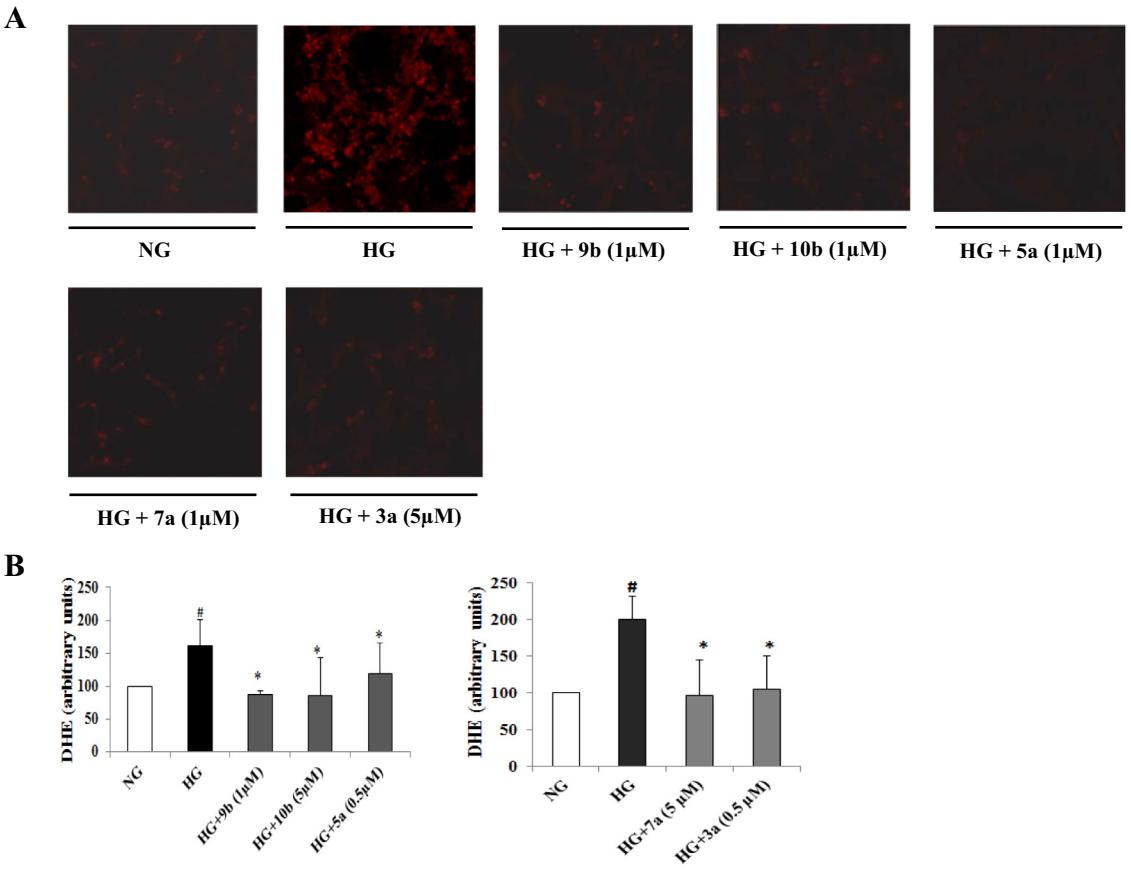

Figure 3. Reduction of high-glucose induced reactive oxygen species (ROS) production. (A) DHE staining of rat glomerular mesangial cells in the presence or absence of **9b**, **10b**, **5a**, **7a**, and **3a**. (B) Mean fluorescence intensity of the digitized images. $^{\#}P$ <0.05 versus NG, $^{*}P$ <0.05 versus HG.

4b–**4f**. Most of the cyclopentanone derivatives were synthesized following reported procedures.[27,30] The condensation reaction was performed in a sealed tube at 90 °C in ethanol or methanol and in the presence of a catalytic amount of trifluoroacetic acid (TFA) according to a modified procedure[31] to afford 18 pyrimidinyl acylhydrazones which were synthesized for the first time and characterized by ^{1}H and ^{13}C NMR and HRMS (ESI).

The effect of the nucleoside analogs was first assessed on fibronectin expression in cultured rat mesangial cells incubated for 48 h with high-glucose (HG: 25 mM) in the presence or absence of the acylhydrazone derivative (Tables 1 and 2).

Several compounds reduced fibronectin expression induced by high glucose treatment (**9b**, **10b**, **3a**, **3c**, etc.). MTT assays were also performed for all of the listed compounds. Several studies have

recently indicated that in the early stages of diabetic nephropathy, mesangial cells undergo a short phase of increased proliferation after which the cells are arrested at the G0 phase and start the accumulation of extra cellular matrix proteins.[32,33] Consequently, the most active thymine and uracil derivatives were further studied by determining their effect on rat glomerular mesangial cell-proliferation and only those that were able to reduce fibronectin expression as well as cellular proliferation induced by high glucose were chosen for further analysis. For example, compounds such as **7c**, **8b**, and **6a** had no significant effect on cellular proliferation and only a few manifested significant effects to be further evaluated as potent inhibitors of renal cellular injury in the diabetic milieu (Fig. 1A and B). Thus, cultured rat glomerular mesangial cells were incubated with or without high-glucose (HG: 25 mM) in the presence or absence of the tested compounds for the assessment of cellular proliferation using the MTT assay. Our results show that these compounds (**9b**, **10b**, **5a**, **7a** and **3a**) were also able to significantly decrease the proliferation of mesangial cells induced by high glucose treatment (Fig. 2).

Several studies stress the role of ROS as major players in cellular injury including Diabetic Nephropathy. More specifically, ROS production has been proven to occur under diabetic conditions.[11,27] However, this effect was clearly attenuated in the presence of nucleoside analogs 9b, 10b, 5a, 7a and 3a (Fig. 3A and B).

In conclusion, we have reported the synthesis of a second series of carbocyclic uridyl, thymidyl and cytidyl acylhydrazone derivatives and their activity in diabetic nephropathy. In contrast to the cytidyl analogs, the thymidyl and uridyl ones displayed significant reduction in protein expression on (HG)-induced mesangial cells. This trend was also observed in the previously reported series of carbanucleosides.[27] This study clearly validates the importance of carbocyclic pyrimidine derivatives for the treatment of diabetes. Furthermore, this is the second reported study highlighting the bioactivity of acylhydrazones in diabetes. However, further studies are called for to test the effect of varying the substituents and refining the length of the linker between the pyrimidine group and the acylhydrazone moiety.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.12.042.

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Update

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Corrigendum

Corrigendum to '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' [Bioorg. Med. Chem. Lett. 26 (2016) 1020–1024]

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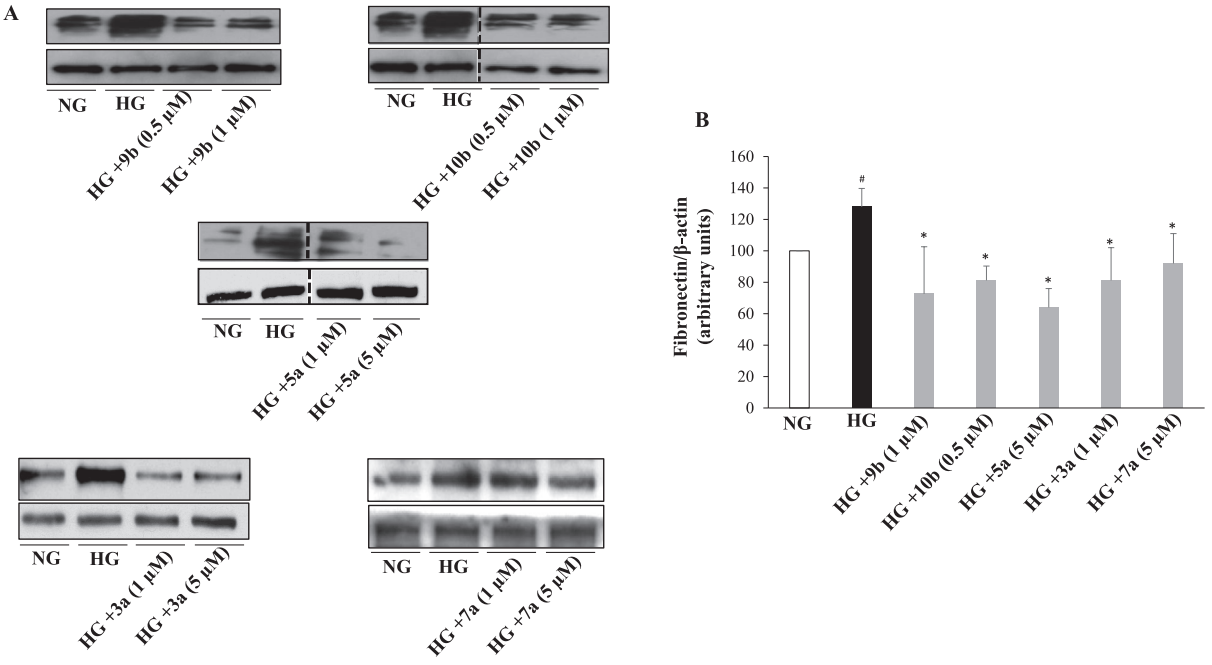
The authors regret that there was an error in Figure 1 of the above article. The correct figure can be found below.



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