Full Paper

Synthesis and Evaluation of 1,ω-Bis(1,2,3,5-thiatriazol-5-yl)alkanes as *In Vitro* and *In Vivo* α-Amylase and Lipase Inhibitors

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Thionyl chloride reacts with 1,ω-bis-(1-tosylamidrazone)alkanes **1** to give a series of 1,ω-bis-(4-alkyl-2-tosyl-1,2,3,5-thiatriazol-5-yl)alkanes **2**. All the newly synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, elemental analysis, and ESI–MS spectral data. All the new compounds were screened for their inhibitory effect on key enzymes related to diabetes and obesity, such as α-amylase and lipase. *In vitro* and *in vivo* studies revealed that these thiatriazole derivatives exert an inhibitory action against these key enzymes. Moreover the administration of these compounds to surviving diabetic rats induced a significant decrease in plasma glucose level. Additively **2d** significantly protected the liver–kidney functions and modulated lipid metabolism, which were evidenced by the decrease in aspartate transaminase (AST), alanine transaminase (ALT), and gamma-glutamyl transpeptidase (GGT) activities and creatinine, urea albumin, LDL-cholesterol and triglycerides levels as well as an increase in the HDL-cholesterol level in surviving diabetic rats. Overall, the findings of the current study indicate that **2d** exhibits attractive properties and can, therefore, be considered for future application in the development of anti-diabetic and hypolipidemic drugs.

Keywords: α-Amylase / 1,ω-Bis-(thiatriazol-5-yl)alkanes / Diabetes / Lipase / Obesity

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Introduction

Diabetes mellitus is a major and growing public health problem throughout the world, with an estimated worldwide prevalence in 2008 of more than of 347 million people. This disease is a heterogeneous disorder with varying incidence and it is reported to constitute the 16th leading cause of global mortality [1]. It is generally recognized that patients with diabetes are at risk for numerous severe complications, including diabetic hyperlipidemia, liver–kidney complications, and hypertension [2–4]. One of the therapeutic approaches for decreasing hyperglycemia and obesity is to retard absorption of glucose by the inhibition of carbohydrate-hydrolyzing enzymes such as α-amylase and α-glucosidase, in the digestive organs [5, 6]. Screening of α-glycosidase inhibitors is becoming increasingly attractive because α-glycosidases are responsible for the processing and hydrolysis of complex carbohydrates into absorbable simple sugar in the small intestine [2–5]. Inhibitors of these enzymes are important molecular tools for glycobiology and can be used to modulate cellular functions.

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On the other hand, five-membered heterocyclic compounds, natural as well as synthetic, are important for their biological activities. Compounds with thiatriazole rings are of interest due to their broad spectrum of biological activities, such as antihypertensive [7], antibacterial [8], antifungal [9], antitubercular [10], fungicidal [11], and anticancer activities [12]. Further, it has been reported that many biologically active natural and synthetic products have interesting molecular symmetry [13]. Recently, some bis-triazole derivatives endowed with antibacterial activities have been reported from our laboratory [14]. Prompted by these observations and in continuation of our search toward the synthesis of new heterocyclic compounds with anticipated biological activities, we synthesized a new series of compounds containing two thiatriazoles rings and investigated the therapeutic action of these compounds on the diabetic status as well as the activities of α-amylase and lipase with respect to lipid profile and liver–kidney toxicity in alloxan induced diabetic rats.

Results and discussion

Chemistry

Bis-amidrazones **1** were prepared according to the procedure described in the literature [14]. These compounds have four nucleophilic centers at positions 1,4 and 1′,4′ that are very reactive face to compounds containing an electrophilic center (e.g., thionyl chloride). In fact, the addition, in cold conditions of two equiv. of thionyl chloride in dichloromethane with one equiv. of **1**, in the presence of a slight excess of anhydrous pyridine afforded, with a good yield, a cyclized product identified as 1,ω-bis-(4-alkyl-2-tosyl-1,2,3,5-thiatriazol-5-yl)alkanes **2** (Scheme 1).

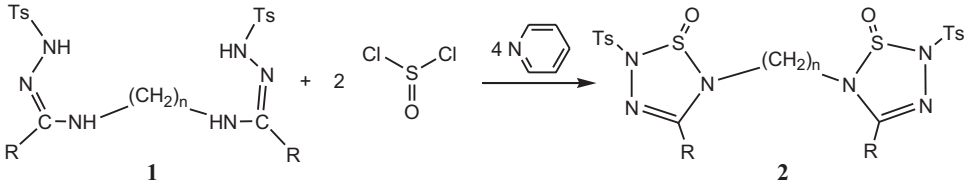
The characterisation data of 1,ω-bis-(4-alkyl-1-oxo-2-tosyl-1,2,3,5-thiatriazol-5-yl)alkanes **2a–g** is presented in the Experimental section. The formation of the cyclised products **2a–g** was confirmed by IR, ^{1}H NMR, ^{13}C NMR, elemental analysis, and ESI–MS. Assignment of selected characteristic IR bands provides significant indications for the formation of bis-thiatriazoles **2a–g**. The absence of the band in the range of 3100–3250 cm^{-1} confirmed the disappearance of the amino groups, while the appearance of an intense band at around 1155 cm^{-1} revealed the ν (S=O) stretch of thiatriazole rings; the absorption band in the range of 1590–1610 cm^{-1} was attributed to the vibration of the –C=N group. ^{1}H NMR spectra of compounds **2a–g** performed in $CDCl_3$ showed the absence of the characteristics signals of NH groups at 6.5 and 8.7 ppm observed in the parent bis-amidrazones **1**. Interestingly, as shown by ^{1}H, ^{1}H COSY experiment (Fig. 1), the N-C*H*$_2$ resonance signals appeared as an ABm system located between 3.30 and 3.80 ppm, indicating the non-equivalence of these two protons, with respect to their hindered environment. ^{13}C NMR spectra showed resonance signals in the range of 147–150 ppm corresponding to the amidino carbons. Elemental analyses were consistent with the proposed formula.

In summary, all the synthesized compounds exhibited satisfactory spectral data consistent with their molecular structures.

Biological activity

α-Amylase and lipase inhibition assay in vitro

Table 1 lists data for an initial series of key enzymes related to diabetes and obesity as α-amylase and lipase inhibitors having substitution on the thiatriazole ring. While all of these compounds have IC_{50} values as α-amylase and lipase inhibitors, relatively little variation in potency is observed. It is noteworthy that compounds **2b**, **2d**, and **2g**, the three examples displaying a CH_2CH_3 substitution on the ring increase the inhibitory action of these drugs against the



Compound	R	n	Compound	R	n
1a, 2a	CH_3	6	**1e, 2e**	CH_3	10
1b, 2b	C_2H_5	6	**1f, 2f**	CH_3	12
1c, 2c	CH_3	8	**1g, 2g**	C_2H_5	12
1d, 2d	C_2H_5	8			

Scheme 1. Synthetic pathway of 1,ω-bis-thiatriazoles derivatives **2a–h** from 1,ω-bis-amidrazones **1a–h**.

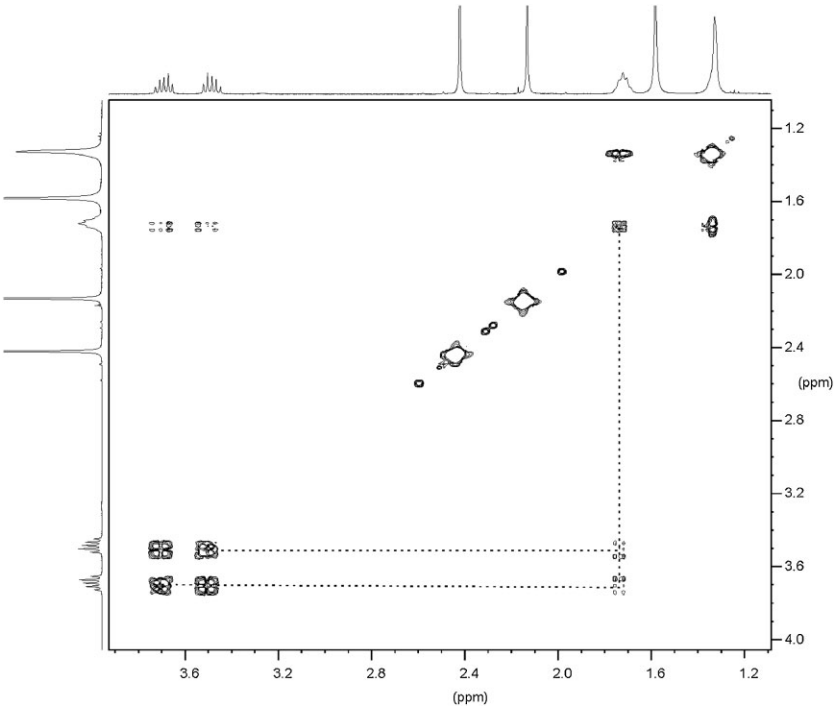


Figure 1. Partial ^{1}H, ^{1}H COSY spectrum of **2c** ($CDCl_3$, 400 MHz, rt).

two enzymes. The largest inhibition of the activity of α-amylase and lipase was observed in **2d**, agreed by the smaller IC_{50} *in vitro* and explaining the use of this compound for the *in vivo* study.

α-Amylase and lipase activities in small intestine of control and treated diabetic rats

The results revealed that diabetes induced a considerable increase in the α-amylase and lipase activities in both the small intestine and the plasma, which leads to a significant increase of plasma glucose, TC and LDL-C and decrease in HDL-C of diabetic rats. On the other hand, in **2d** treated diabetic rats, the activities of those enzymes underwent considerable improvements, which leads to decrease of the glucose, TG and LDL-C and increase in LDH-C concentration in the plasma of surviving diabetic rats (Fig. 2).

Liver functions of control and treated diabetic rats

Table 2 shows that the administration of **2d** to surviving diabetic rats seems to have reverted back this increase and ameliorated all indices related to liver dysfunction induced by diabetes. Further histological analyses confirmed the positive effect of **2d**. As compared to normal rats (Fig. 3A) and to diabetic rats characterized by apparition of fatty cysts (Fig. 3B), the histopathological study of **2d**-treated diabetic rats livers showed a neat decrease of the latter (Fig. 3D). However, the administration of **2d** to surviving diabetic rats protects liver tissues.

Table 1. *In vitro* α-amylase and lipase inhibition assay of thiatriazoles.

Compound	R	n	IC_{50} in μg/mL (μM) against α-amylase	Compound	IC_{50} in μg/mL (μM) against lipase
Acarbose (645.6)			14.88 (23.05)	Orlistat (495.73)	35.21 (71.0)
2a (655.82)	CH_3	6	94.32 (143.8)		115.8 (176.5)
2b (656.86)	C_2H_5	6	84 (127.8)		108.8 (165.6)
2c (656.86)	CH_3	8	82.96 (126.3)		112.2 (170.8)
2d (684.91)	C_2H_5	8	65.16 (95.1)		74.6 (108.9)
2e (702.92)	CH_3	10	94.22 (134.0)		98.1 (139.5)
2f (712.97)	CH_3	12	91.33 (128.1)		121.5 (170.4)
2g (759.03)	C_2H_5	12	69.25 (91.2)		135.1 (178.0)

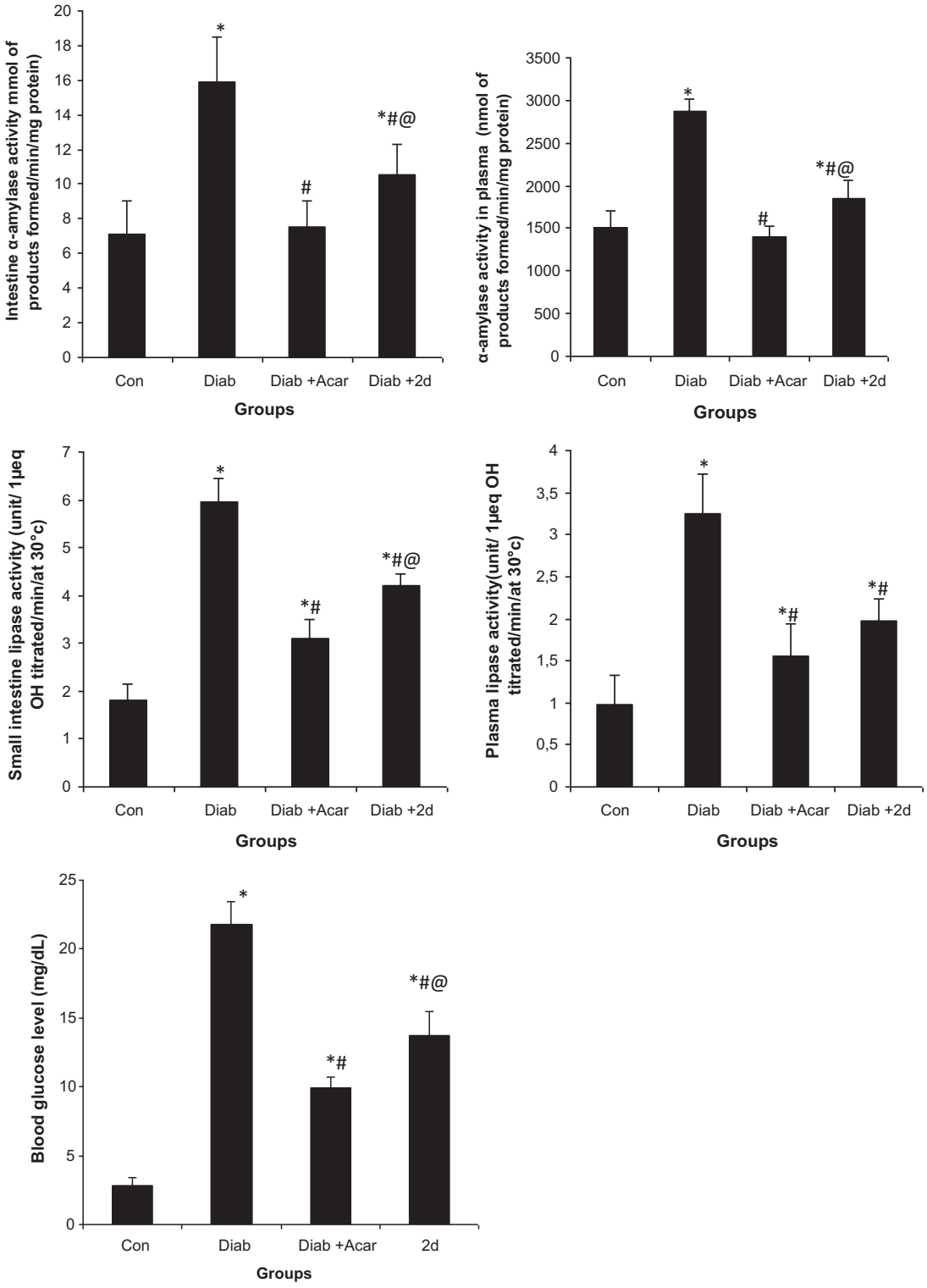


Figure 2. α-Amylase and lipase activities and plasma blood glucose level of control and experimental groups of rats. Values are statistically presented as follows: $^{*}p < 0.05$ significant differences compared to controls. $^{\#}p < 0.05$ significant differences compared to diabetic rats $^{@}p < 0.05$ significant differences to diabetic rats treated with acarbose (Cont: control; Diab: diabetic; Diab + Acar: diabetic rats treated with acarbose; Diab + **2d**: diabetic rats treated with **2d**).

Table 2. Lipid profile, liver-kidney toxicity indices of control and experimental groups of rats.

Groups	Cont	Diab	Diab+Acar	Diab+ 2d
Serum (mmol/L)				
T-Ch	1.83 ± 0.1	2.64 ± 0.2*	1.97 ± 0.1$^{*\#}$	2.18 ± 0.13$^{*\#@}$
TG	0.95 ± 0.15	1.77 ± 0.14*	1.31 ± 0.08$^{*\#}$	2.15 ± 0.11$^{*\#@}$
LDL-C	0.79 ± 0.11	1.63 ± 0.17*	0.95 ± 0.04$^{*\#}$	0.93 ± 0.02$^{\#}$
HDL-C	0.85 ± 0.09	0.65 ± 0.05*	0.75 ± 0.07$^{\#}$	1.21 ± 0.06$^{*\#@}$
Liver function				
AST	82 ± 6.59	185 ± 11*	88 ± 9$^{\#}$	69 ± 69$^{*\#@}$
ALT	52.5 ± 9.25	133 ± 8.18*	58.3 ± 4.5$^{\#}$	119 ± 14$^{*\#@}$
T-Bili	1.52 ± 0.13	2.58 ± 0.18*	1.63 ± 0.11$^{\#}$	1.78 ± 0.12$^{*\#@}$
D-Bili	0.18 ± 0.01	0.76 ± 0.05*	0.31 ± 0.08$^{*\#}$	0.51 ± 0.04$^{*\#@}$
GGT	3.25 ± 0.5	5.5 ± 0.57*	4 ± 0.81$^{*\#}$	4.12 ± 0.61$^{*\#}$
Kidney function				
Creatinine (μmol/L)	40.8 ± 1.52	49.26 ± 0.25*	43.56 ± 1.26$^{*\#}$	39.5 ± 8.64$^{\#}$
Urea (mmol/L)	6.64 ± 0.35	12.05 ± 1.52*	7.96 ± 0.2$^{*\#}$	8.71 ± 0.54$^{*\#}$
Uric acid (μmol/L)	1.41 ± 5.08	148.79 ± 13.81*	55.88 ± 2.04$^{*\#}$	59.6 ± 6.69$^{*\#}$

Values are statistically presented as follows: $^{*}p < 0.05$ significant differences compared to controls. $^{\#}p < 0.05$ significant differences compared to diabetic rats. $^{@}p < 0.05$ significant differences to diabetic rats treated with acarbose (Cont: control; Diab: diabetic; Diab + Acar: diabetic rats treated with acarbose; Diab + **2d**: diabetic rats treated with **2d**).

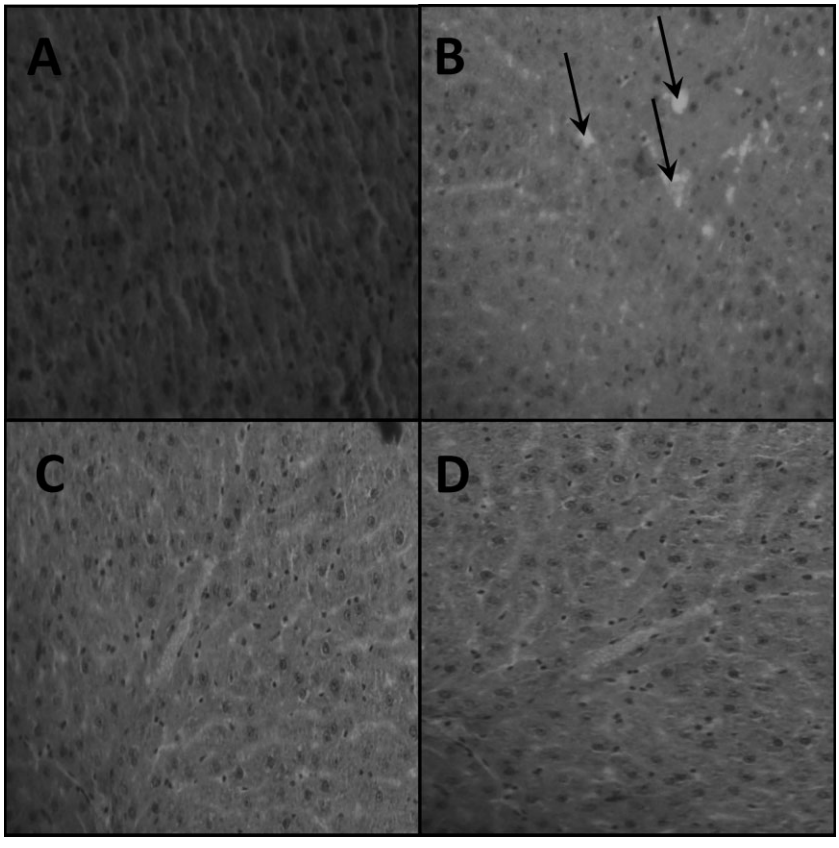


Figure 3. Histopathological studies of liver in the control and experimental groups of rats. Section of the liver from a control rat showing normal architecture (A); liver of diabetic rat showing fatty cysts apparition in liver tissues (B). Liver of diabetic rat treated with acarbose (C) or **2d** (D): protective effect is observed in evidence by the absence of fatty cysts.

Kidney functions of control and treated diabetic rats

Table 2 evidenced that the administration of **2d** to surviving diabetic rats seems to have reverted back this increase of plasma creatinine, urea, and uric acid as compared to untreated diabetic rats. The positive effect of **2d** was confirmed by histological analyses. In fact, in kidneys of diabetic rats showing glomerular hypertrophy as compared to control rat (Fig. 4A), administration of acarbose (Fig. 4C) and **2d** (Fig. 4D) to surviving diabetic rats showed a potential protective action, evidenced by the reversion of glomerular size to normal.

Conclusion

In summary, we prepared and evaluated a series of novel 1,ω-bis-(4-alkyl-2-tosyl-1,2,3,5-thiatriazol-5-yl)alkanes for their inhibitory effect of key enzymes related to diabetes and obesity. Most of the compounds showed significant inhibitory action against α-amylase and lipase, two enzymes implicated in the development of diabetes and obesity. In particular, the compound **2d** showed promising inhibitor activity against the two enzymes. It can allow to explore new pathways for the treatment and prevention of diabetes and related diseases, and can be considered as a potential therapeutic agent for the treatment of diabetes, cardiovascular diseases, and liver–kidney dysfunction. For these reasons, further studies are currently under progress in our laboratories to more explore this anti-diabetic agent and to make its application suitable for pharmaceutical and medicinal application for the development of anti-diabetic and hypocholesterolemic drugs.

Experimental

Chemistry

Melting points were determined with an Electrothermal 9100 apparatus and are uncorrected. IR spectra were recorded in KBr pellets (ν in cm^{-1}), on a Perkin–Elmer 100 infrared spectrophotometer whose precision is of 2 cm^{-1} covering 400–4000 cm^{-1}. The NMR spectra were recorded in $CDCl_3$ on a Bruker Avance spectrometer (300 MHz for ^1H, 75 MHz for ^{13}C). ^1H and ^{13}C chemical shifts are given on the δ scale (ppm) and are referenced to internal TMS. The multiplicities of the signals are indicated by the following abbreviations: s: singlet, d: doublet, t: triplet, q: quadruplet, m: multiplet and coupling constants are expressed in Hertz (Hz). Mass spectra were acquired on a Bruker micrOTOF-Q apparatus using electrospray ion trap (ESI) technique by collision-induced dissociation (in positive or negative mode). Elemental analyses were performed on a Thermo Finnigan EA 1112 apparatus, both at the Service Commun d'Analyses, Université de Lorraine, Nancy.

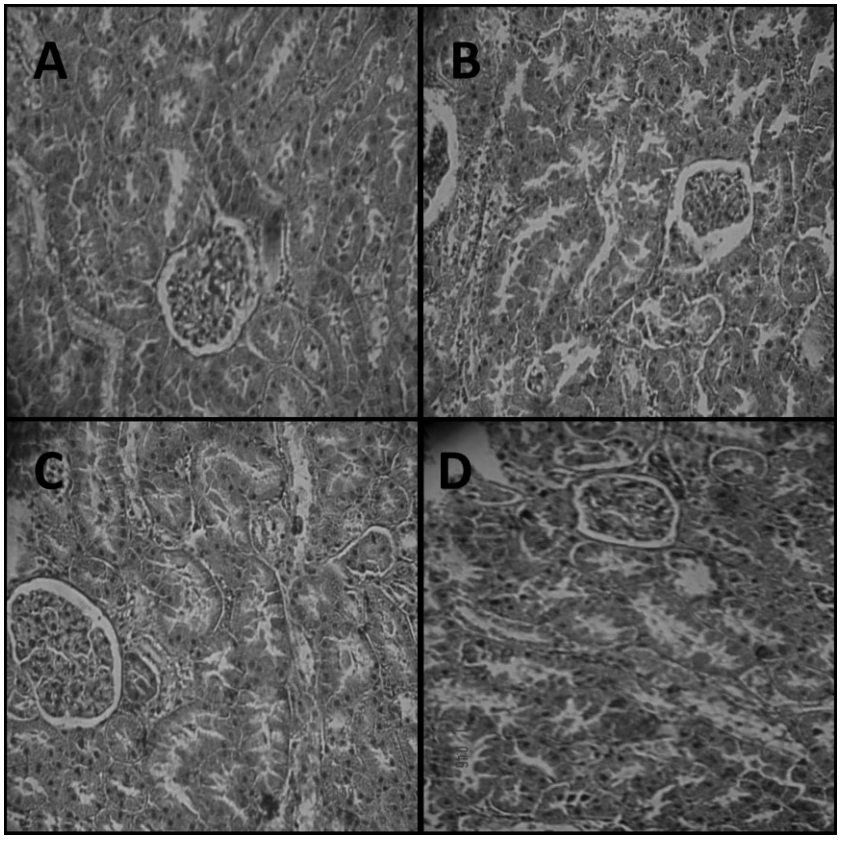


Figure 4. (A) Normal rat kidney. (B) Diabetic rat kidney: tubular epithelial damage messangial and capillary proliferation. Kidney of diabetic rat treated with acarbose (C) or **2d** (D).

The reactions and the purity of substances were monitored by thin layer chromatography (TLC) (elution systems chloroform/ethanol, 9:1) using aluminum sheets with silica gel 60 F_{254} Merck. All reactions, unless otherwise stated, were carried out under nitrogen atmosphere in dry solvents under anhydrous conditions. Bis-amidrazones **1** were synthesized according to the literature procedures [14]. All other reagents were purchased and used without purification.

*Synthesis of bis-(4-alkyl-1-oxo-2-tosyl-1,2,4,3-thiatriazol-5-yl)alkanes **2a–g***

To a solution of 1.0×10^{-3} mole of bis-amidrazone **1** and 4.0×10^{-3} mole of pyridine with 20 mL of anhydrous dichloromethane (CH_2Cl_2), stirred magnetically in ice-cold water (H_2O), was added dropwise a solution of 2.0×10^{-3} mole of thionyl chloride ($SOCl_2$) with 10 mL of anhydrous dichloromethane. The mixture was stirred during 48 h (TLC monitoring, SiO_2, chloroform ($CHCl_3$)/ethanol (C_2H_5OH) 9:1), then was washed three times with 20 mL of distillated water. The dichloromethane layer was recovered, dried and the solvent was evaporated to give the resulting compound **2** precipitated soon by the addition of diethyl ether. After filtration, the resulting compounds were re-crystallized from methanol (CH_3OH).

*1,6-Bis-(4-methyl-1-oxo-2-tosyl-1,2,3,5-thiatriazol-5-yl)-hexane (**2a**)*

Yield: 55%. Mp: 174–176°C. IR: 1156 (S=O), 1696 (C=N); ^{1}H NMR ($CDCl_3$): δ 1.35 (m, 4 H, CH_2), 1.71 (m, 4 H, CH_2), 2.11 (s, 6 H, 2 CH_3), 2.40 (s, 6 H, CH_3 Ts), 3.49–3.65 (ABm, 4 H, CH_2-N), 7.29 (d, 4 H, J = 8.4 Hz, Ar*H* Ts), 7.87 (d, 4 H, J = 8.4 Hz, Ar*H* Ts). ^{13}C NMR ($CDCl_3$): 11.2 (*C*H_3), 21.8 (*C*H_3 Ts), 26.2, 30.2 (*C*H_2), 43.2 (N*C*H_2), 128.6, 129.7, 134.6, 145.5 (*C* Ar), 149.2 (*C*(4)). Anal. calcd. for $C_{24}H_{32}N_6O_6S_4 \cdot H_2O$ (655.82): C 43.95; H 5.22; N 12.81; found: C 44.13; H 5.16; N 12.79. ESI–MS (pos. mode): 629.13 $[M+H^+]^+$, 651.11 $[M+Na^+]^+$, 667.08 $[M+K^+]^+$.

*1,6-Bis-(4-ethyl-1-oxo-2-tosyl-1,2,3,5-thiatriazol-5-yl)-hexane (**2b**)*

Yield: 60%. Mp: 160–162°C. IR: 1147 (S=O),1595 (C=N); ^{1}H NMR ($CDCl_3$): 1.20 (t, 3 H, J = 6.8 Hz, CH_3), 1.35 (m, 4 H, CH_2), 1.71 (m, 4 H, CH_2), 2.38 (m, 10 H, CH_3 Ts and CH_2CH$_3$), 3.50–3.61 (ABm, 4 H, CH_2–N), 7.30 (d, 4 H, J = 7.8 Hz, Ar*H* Ts), 7.87 (d, 4 H, J = 7.8 Hz, Ar*H* Ts). ^{13}C NMR ($CDCl_3$): 10.5 (CH_2CH_3), 19.6 (CH_2CH_3), 21.8 (*C*H_3 Ts), 26.2, 30.2 (*C*H_2), 42.7 (N*C*H_2), 128.6, 129.6, 134.7, 145.3 (*C* Ar), 153.2 (C(4)). Anal. calcd. for $C_{26}H_{36}N_6O_6S_4$ (656.86): C 47.54; H 5.52; N 12.79; found: C 47.74; H 5.55; N 12.96. ESI–MS (pos. mode): 657.16 $[M+H^+]^+$, 679.15 $[M+Na^+]^+$, 695.12 $[M+K^+]^+$.

*1,8-Bis-(4-methyl-1-oxo-2-tosyl-1,2,3,5-thiatriazol-5-yl)-octane (**2c**)*

Yield: 67%. Mp: 136–138°C. IR: 1170 (S=O), 1595 (C=N). ^{1}H NMR ($CDCl_3$): 1.32 (m, 4 H, CH_2), 1.58 (m, 4 H, CH_2), 1.72 (m, 4 H, CH_2), 2.13 (s, 6 H, CH_3), 2.42 (s, 6 H, CH_3 Ts), 3.48–3.69 (ABm, 4 H, CH_2–N), 7.31 (d, 4 H, J = 8.0 Hz, Ar*H* Ts), 7.91 (d, 4 H, J = 8.0 Hz, Ar*H* Ts). ^{13}C NMR ($CDCl_3$): 11.9 (*C*H_3), 21.7 (*C*H_3 Ts), 26.4, 28.8, 30.3 (*C*H_2), 43.2 (N*C*H_2), 128.6, 129.6, 134.7, 145.3 (*C* Ar), 149.0 (C(4)). Anal. calcd. for $C_{26}H_{36}N_6O_6S_4$ (656.86): C 47.54; H 5.52; N 12.79; found: C 47.78; H 5.58; N 12.82. ESI–MS (pos. mode): 657.16 $[M+H^+]^+$, 679.15 $[M+Na^+]^+$, 695.12 $[M+K^+]^+$.

*1,8-Bis-(4-ethyl-1-oxo-2-tosyl-1,2,3,5-thiatriazol-5-yl)-octane (**2d**)*

Yield: 63%. Mp: 144–146°C. IR: 1151 (S=O), 1598 (C=N). ^{1}H NMR ($CDCl_3$): 1.24 (m, 14 H, CH_2CH_3 and CH_2), 1.71 (m, 4 H, CH_2), 2.38 (s, 10 H, CH_2CH$_3$ and CH_3 Ts), 3.49–3.65 (ABm, 4 H, CH_2–N), 7.31 (d, 4 H, J = 7.8 Hz, Ar*H* Ts), 7.89 (d, 4 H, J = 7.8 Hz, Ar*H* Ts). ^{13}C NMR ($CDCl_3$): 10.5 (CH_2CH_3), 19.6 (CH_2CH_3), 21.8 (*C*H_3 Ts), 26.5, 28.8, 30.2 (*C*H_2), 42.8 (N*C*H_2), 128.6, 129.5, 134.6, 145.3 (*C* Ar), 153.3 (C(4)). Anal. calcd. for $C_{28}H_{40}N_6O_6S_4$ (684.91): C 49.10; H 5.89; N 12.27; found: C 48.95; H 5.95; N 12.02. ESI–MS (pos. mode): 685.19 $[M+H^+]^+$, 707.18 $[M+Na^+]^+$, 723.15 $[M+K^+]^+$.

*1,10-Bis-(4-methyl-1-oxo-2-tosyl-1,2,3,5-thiatriazol-5-yl)-decan (**2e**)*

Yield: 66%. Mp: 124–126°C. IR: 1596 (C=N), 1152 (S=O). ^{1}H NMR ($CDCl_3$): 1.21 (m, 12 H, CH_2), 1.63 (m, 4 H, CH_2), 2.05 (s, 6 H, CH_3), 2.34 (s, 6 H, CH_3 Ts), 3.43–3.59 (ABm, 4 H, CH_2–N), 7.23 (d, 4 H, J = 8.1 Hz, Ar*H* Ts), 7.81 (d, 4 H, J = 8.1 Hz, Ar*H* Ts); ^{13}C NMR ($CDCl_3$): 11.4 (*C*H_3), 21.3 (*C*H_3 Ts), 26.0, 28.4, 28.7, 39.8 (*C*H_2), 42.7 (N*C*H_2), 128.1, 129.1, 134.1, 144.8 (*C* Ar), 148.7 (*C*(4)). Anal. calcd. for $C_{28}H_{40}N_6O_6S_4 \cdot H_2O$ (702.92): C 47.84; H 6.02; N 11.95; found: C 47.94; H 5.86; N 11.82. ESI–MS (pos. mode): 685.19 $[M+H^+]^+$, 707.17 $[M+Na^+]^+$, 723.15 $[M+K^+]^+$.

*1,12-Bis-(4-methyl-1-oxo-2-tosyl-1,2,3,5-thiatriazol-5-yl)-dodecane (**2f**)*

Yield: 70%. Mp: 140–142°C. IR: 1598 (C=N), 1154 (S=O), ^{1}H NMR ($CDCl_3$): 1.23 (m, 16 H, CH_2), 1.65 (m, 4 H, CH_2), 2.12 (s, 6 H, CH_3), 2.35 (s, 6 H, CH_3 Ts), 3.45–3.62 (ABm, 4 H, CH_2–N), 7.21 (d, 4 H, J = 7.8 Hz, Ar*H* Ts), 7.88 (d, 4 H, J = 7.8 Hz, Ar*H* Ts). ^{13}C NMR ($CDCl_3$): 11.9 (*C*H_3), 21.7 (*C*H_3 Ts), 26.6, 29.0, 29.3, 29.7, 30.3 (*C*H_2), 43.3 (N*C*H_2), 128.6, 129.6, 134.8, 145.2 (C Ar), 149.1 (*C*(4)). Anal. calcd. for $C_{30}H_{44}N_6O_6S_4$ (712.97): C 50.54; H 6.22; N 11.79; found: C 50.96; H 6.28; N 11.85. ESI–MS (pos. mode): 713.23 $[M+H^+]^+$, 735.21 $[M+Na^+]^+$, 751.18 $[M+K^+]^+$.

*1,12-Bis(4-ethyl-1-oxo-2-tosyl-1,2,3,5-thiatriazol-5-yl)-dodecane (**2g**)*

Yield: 67%. Mp: 133–135°C. IR: 1598 (C=N), 1153 (S=O). ^{1}H NMR ($CDCl_3$): 1.23 (m, 16 H, CH_2CH_3 and CH_2), 1.71 (m, 4 H, CH_2), 2.41 (m, 10 H, CH_2CH_3 and CH_3 (Ts)), 3.47–3.66 (ABm, 4 H, CH_2–N), 7.29 (d, 4 H, J = 8.1Hz, Ar*H* Ts), 7.91 (d, 4 H, J = 8.1 Hz, Ar*H* Ts). ^{13}C NMR ($CDCl_3$): 10.5 (*C*H_3), 19.6 (CH_2CH_3), 21.8 (*C*H_3 Ts), 26.6, 29.0, 29.3, 29.7, 30.3 (*C*H_2), 42.8 (N*C*H_2), 128.6, 129.6, 134.8, 145.23 (*C* Ar), 153.3 (*C*(4)). Anal. calcd. for $C_{32}H_{48}N_6O_6S_4 \cdot H_2O$ (759.03): C 50.63; H 6.63; N 11.07; found: C 50.63; H 6.66; N 11.27. ESI–MS (pos. mode): 741.26 $[M+H^+]^+$, 763.24 $[M+Na^+]^+$, 779.21 $[M+K^+]^+$.

Biology

Materials

Porcine lipase and alpha-amylase, 4-methylumbelliferyl oleate (4-MU oleate) and starch were obtained from Sigma (St. Louis, MO, USA). Alloxan, was purchased from Sigma–Aldrich (St. Louis, MO, USA), the GOD, HDL, TC, TG, aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transpeptidase (GGT), T-bili and D-bili were from Biomaghreb analyticals (Tunis, Tunisia). Orlistat and acarbose were purchased from

Central Pharmacy (Sfax, Tunisia). All other chemicals used were of analytical grade.

Animals and treatments

The assays of the present study were conducted on adult male Wistar rats, weighing 212 ± 19 g, which were obtained from the local Central Pharmacy, Tunisia. All rats were kept in an environmentally controlled breeding room (temperature: 20 ± 2°C, humidity: 60 ± 5%, 12-h dark/light cycle) where they had standard diets and free access to tap water. The experimental protocols were conducted in accordance with the guide for the care and use of laboratory animals issued by the University of Sfax, Tunisia, and approved by the Committee of Animal Ethics.

Diabetes was induced in rats by a single intraperitoneal injection of freshly prepared alloxan solution in normal saline at a dose of 150 mg/kg body weight [6]. After 2 weeks, the rats whose blood glucose was higher than 2 g/L were chosen for the experiment. On the day the experiments started, and before treatment, the rats were divided into four groups of eight animals, each as follows:

Group 1: diabetic control rats at day 30 (Diab30)
Group 2: diabetic rats treated with acarbose by gastric gavage route food (10 mg/kg of body weight/day during 30 days) and termed [Diab–Acar].
Group 3: diabetic rats treated with **2d** by gastric gavage route food (65 mg/kg of body weight/day during 30 days) and termed [Diab–**2d**].
Group 4: normal rats were used as controls, were scarified at day 30, considered as referent non-diabetic rats at day 30 (Con30).

One month later, the rats were weighed and sacrificed by decapitation, and their trunk blood collected. The serum was prepared by centrifugation (1500 × g, 15 min, 4°C).

α-*Amylase assay in vitro*

The *in vitro* α-amylase inhibition activity of all drugs was determined based on the spectrophotometric assay using acarbose as the reference compound. The drugs were dissolved in DMSO to give concentrations of 25, 50, and 100 μg/mL. The enzyme α-amylase solution was prepared by mixing 3.246 mg of α-amylase in 100 mL of 40 mM phosphate buffer, pH 6.9. Positive control, acarbose was obtained by dissolving 50 mg in 50 mL of phosphate buffer and diluted to get a concentration of 2.5 μg/mL. The essays were conducted by mixing 80 μL of plant extract, 20 μL of α-amylase solution and 1 mL of 2-chloro-4-nitrophenol-α-D-maltotrioside (CNPG3) [16]. The mixture was incubated at 37°C for 5 min. The absorbance was measured at 405 nm. Similarly, a control reaction was carried out without the drugs/acarbose. Percentage inhibition (PI) was calculated by the expression:

$$\mathrm{PI} = \frac{\mathrm{Absorbance_{Control}} - \mathrm{Absorbance_{test}}}{\mathrm{Absorbance_{control}}} \times 100$$

Lipase assay in vitro

The method was modified from the assay reported by Junge et al. [17], in which triolein was used as a substrate to measure the pancreatic lipase inhibitory activity of all samples. Briefly, the reaction mixture (3.06 mL) containing 135 mM triolein emulsified in sodium thioglycolate, lipase (0.4 unit) and the sample was adjusted to pH 8.8, incubated at 25°C. The inhibitory activity of the sample was calculated from the titrant volume. This was done in triplicate for each experiment.

$$\mathrm{PI} = \frac{\mathrm{Absorbance_{Control}} - \mathrm{Absorbance_{test}}}{\mathrm{Absorbance_{control}}} \times 100$$

Biochemical analysis

The mucosal small intestine of each rat was excised and the lumen was flushed out several times with 0.9% NaCl. The mucosal washing and the scraped mucosa were pooled, homogenized, and centrifuged (5000 × *g*, 15 min). The supernatant was frozen and stored at −80°C for further use in subsequent enzymatic assays. The activity of α-amylase was obtained by measuring the amount of glucose released from starch substrate [15]. Lipase activity was assayed, with olive oil as substrate, using the method described by Tietz and Fiereck [16]. For histochemical procedures, tissue specimens of the liver and kidney were obtained and fixed with 10% buffered formalin, and subsequently embedded in paraffin. After that, the paraffin-embedded samples were cut in sections (thickness, 5 μm) and then stained with hematoxylin–eosin. The samples were then examined using an Olympus CX41 light microscope.

Statistical analysis

Data are presented as means ± SD. Determinations were performed from eight animals per group and differences were examined by a one-way analysis of variance (ANOVA) followed by the Fisher test (Stat View). The significance was accepted at $p < 0.05$.

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