Research Article

Synthesis of Potential agents for the Therapy of Type 2 Diabetes Mellitus Based on Thietane Containing 2-Bromoimidazole-4,5-Dicarboxylic acid derivatives

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Received: 29-11-2016; **Revised:** 10-01-2017; **Accepted:** 24-01-2017.

ABSTRACT

Thietane containing 2-bromoimidazo[4,5-d]pyridazine-4,7(*5H,6H*)-diones and 2-bromo-1-(thietan-3-yl)-, 2-bromo-1-(1-oxothietan-3-yl)-, and 2-bromo-1-(1,1-dioxothietan-3-yl)imidazole-4,5-dicarboxyic acid hydrazides and diylidenhydrazides were synthesized. These compounds were investigated for the effect on the formation of advanced glycation end-products, for deglycation activity, and for inhibition of dipeptidyl peptidase-4, glycogen phosphorylase, and α-glycosidase *in vitro*. Newly identified inhibitors of advanced glycation end-products and α-glycosidase were found to be comparable in activity to the reference agents, aminoguanidine and acarbose, respectively.

Keywords: Imidazole-4,5-dicarboxylic acid, imidazo[4,5-*d*]pyridazine-4,7(5*H*,6*H*)-dione, thietane, hydrazide, diabetes mellitus.

INTRODUCTION

Imidazole derivatives are biologically active compounds with a broad range of action[1]. Particularly, imidazole-4,5-dicarboxylic acid derivatives hold high promise, as some of them were found to exhibit antiviral activity[2,3], 1-methylimidazole-4,5-dicarbohydrazide inhibits monoaminooxidase[4], hydrazinium imidazole-4,5-dicarboxylate shows antimicrobial activity[5], and imidazole-4,5-dicarboxylic acid hydrazides and diylidenhydrazides also have antimicrobial and insecticidal activities[6]. A computer prediction of the biological activities of imidazole-4,5-dicarbohydrazides showed good prospects for the search for agents to treat type 2 diabetes mellitus among these derivatives. For the search for new biologically active compounds, we prepared new thietane containing 2-bromoimidazole-4,5-dicarboxylic acid derivatives.

MATERIALS AND METHODS

General

^{1}H NMR spectra were recorded on Bruker AM-300 and Bruker Avance III instruments operating for protons at 300 and 500 MHz, respectively. The residual solvent signals were used as the internal standards. TLC was carried out on Sorbfil plates with dioxane (IVa, IVb, IVd) or a 1:1 dioxane-ethanol mixture (IVc) as the mobile phase. Melting points were determined on a Stuart SMP30 apparatus. The elemental analysis data for C, H, and N of the products corresponded to the calculated values.

Compounds Ia-c, IIa-c, and IIIa-c were prepared by procedures reported in[7].

2-Bromo-1-(thietan-3-yl)imidazole-4,5-dicarboxylic acid di(methyl ethylidenehydrazide) (IVa).

Compound IIa (0.37 g, 1.10 mmol) was dissolved in a mixture of 2 mL of dilute HCl and 15 mL of ethanol, and 5 mL of acetone was added. The reaction mixture was stirred for 15 min at room temperature. The precipitate was filtered off, washed with ethanol, and dried. The product was recrystallized from benzene to give 0.22 g (49%) of compound IVa. Mp (°C): 214-216; Rf: 0.40; ^{1}H NMR (DMSO-d_6): 1.72 (s, 3H, *E*-CH_3), 1.87 (s, 3H, *E*-CH_3), 1.89 (s, 3H, *E*-CH_3), 1.91 (s, 3H, *Z*-CH_3), 1.94 (s, 3H, *E+Z*-CH_3), 1.98 (s, 3H, *Z*-CH_3), 1.99 (s, 3H, *Z*-CH_3), 3.34-3.43 (m, 2H, *E+Z*-S$(CH)_2$), 4.05-4.09 (m, 2H, *E*-S$(CH)_2$), 4.19-4.23 (m, 2H, *Z*-S$(CH)_2$), 5.59-5.63 (m, 1H, *E*-NCH), 6.23-6.31 (m, 1H, *Z*-NCH), 9.91 (s, 1H, *E*-NH), 10.42 (s, 1H, *Z*-NH), 11.18 (s, 1H, *E*-NH), 12.22 (s, 1H, *Z*-NH) ppm; CHN analysis for $C_{14}H_{19}BrN_6O_2S$; C 40.49; H 4.61; N 20.24 Found C 40.80; H 4.58; N 20.19.

2-Bromo-1-(thietan-3-yl)imidazole-4,5-dicarboxylic acid di(ethylethylidenehydrazide) (IVb).

Compound IIa (0.50 g, 1.50 mmol) was dissolved in a mixture of 2 mL of dilute HCl and 15 mL of ethanol, and 5 mL of methyl ethyl ketone was added. The reaction mixture was stirred for 1 h at room temperature. The precipitate was filtered off, washed with ethanol, and dried. The product was recrystallized from ethanol to give 0,36 g (54%) of compound IVb. Mp (°C): 188-189; Rf: 0.64; ^{1}H NMR ($CDCl_3$): 1.15-1.22 (m, 6H, $2CH_3$), 2.01 (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 2.39-2.45 (m, 4H, *E*-$2CH_2$ + *Z*-$2CH_2$), 3.35-3.41 (m, 2H, S$(CH)_2$), 4.39-4.45 (m, 2H, S$(CH)_2$), 6.91-7.09 (m, 1H, *E+ Z*-NCH), 10.05 (s, 1H, *E*-NH), 10.19 (s, 1H, *Z*-NH) ppm; CHN analysis for $C_{16}H_{23}BrN_6O_2S$; C 38.99; H 4.44; N 19.48 Found C 39.14; H 4.46; N 19.52.

2-Bromo-1-(1-oxothietan-3-yl)imidazole-4,5-dicarboxylic acid di(methylethylidenehydrazide) (IVc).

Compound IIb (0.40 g, 1.14 mmol) was dissolved in a mixture of 2 mL of dilute HCl and 18 mL of water, and 5 mL of acetone was added. The reaction mixture was stirred for 15 min at room temperature. The product was extracted twice with chloroform (25 mL portions) and then chloroform was evaporated. The product was recrystallized from methanol to give 0.44 g (89%) of compound IVc. Mp (°C): $>250_{decomp.}$; Rf: 0.52; ^{1}H NMR ($CDCl_3$): 2.04 (s, 3H, CH_3), 2.14 (s, 3H, CH_3), 2.16 (s, 3H, CH_3), 3.34-3.42 (m, 2H, $S(CH)_2$), 4.48-4.55 (m, 2H, $S(CH)_2$), 6.63-6.78 (m, 1H, NCH) ppm; CHN analysis for $C_{14}H_{19}BrN_6O_3S$; C 43.34; H 5.23; N 18.96 Found C 43.50; H 5.25; N 19.01.

2-Bromo-1-(1,1-dioxothietan-3-yl)imidazole-4,5-dicarboxylic acid di(methylethylidenehydrazide) (IVd).

Compound IIc (0.57 g, 1.55 mmol) was dissolved in a mixture of 2 mL of dilute HCl and 18 mL of water, and 5 mL of acetone was added. The reaction mixture was stirred for 15 min at room temperature. The precipitate was filtered off, washed with acetone, and dried. The product was recrystallized from acetone to give 0.39 g (56%) of compound IVd. Mp (°C): 237-238; Rf: 0.60; ^{1}H NMR ($CDCl_3$): 2.05 (s, 3H, CH_3), 2.13 (s, 3H, CH_3), 2.14 (s, 3H, CH_3), 2.18 (s, 3H, CH_3), 4.43-4.48 (m, 2H, $S(CH)_2$), 5.27-5.32 (m, 2H, $S(CH)_2$), 6.56-6.63 (m, 1H, NCH), 10.09 (s, 1H, NH), 13.48 (s, 1H, NH) ppm; CHN analysis for $C_{14}H_{19}BrN_6O_4S$; C 37.59; H 4.28; N 18.79 Found C 37.42; H 4.26; N 18.71.

The glycation of proteins with glucose was modeled in a reaction mixture containing glucose (500 mM) and bovine serum albumin (1 mg/mL) dissolved in a phosphate buffer solution (pH 7.4); sodium azide was added in a concentration of 0.02% to prevent bacterial growth[8]. The test compounds were dissolved in DMSO. They were added to experimental samples to final concentrations of $1{\cdot}10^{-3}$ M and $1{\cdot}10^{-4}$ M, while control samples were prepared by adding the same volume of the solvent. All experimental samples were incubated for 24 h at 60 °C. After incubation, specific fluorescence of glycated albumin was determined using a F-7000 spectrofluorimeter (Hitachi, Japan) at λ_{ex} of 370 nm and λ_{em} of 440 nm. The antiglycation activity was calculated relative to the fluorescence of control samples. Aminoguanidine, which is a known inhibitor of non-enzymatic glycosylation, was used as the reference compound[9].

The ability of compounds to break the crosslinks of glycated proteins *in vitro* was determined by a described method[10]. The protein glycation was modeled in the reaction mixture containing glucose (400 mM) and bovine serum albumin (0.8 mg/mL) dissolved in 50 mM phosphate buffer (pH 7.4). The mixture was incubated at 60°C for 40 h. After the incubation, the mixture was dispensed dropwise into Eppendorf tubes (600 µL into each tube), and 60 µL of 100% trichloroacetic acid was added into each tube. After centrifuging at 15000 rpm for 4 min, up to 0.9 mL of 50 mM phosphate buffer (pH 7.4) and 0.1 mL of a test compound were added to the precipitated glycated albumin (reaction volume 1 mL), and the resulting mixture was incubated at 60°C for 40 h. The test compounds were dissolved in DMSO and added to experimental samples to final concentration of 1 mM, while control samples were prepared by adding the same volume of the solvent. After incubation, the precipitated glycated albumin was dissolved in 3 mL of phosphate salt buffer (pH 10.0), and the specific fluorescence of glycated albumin was determined using an Infinite M200 PRO microplate reader (Tecan, Austria) at λ_{ex} 370 nm and λ_{em} 440 nm. The ability of the compounds to break the cross-links of glycated proteins was calculated relative to the fluorescence of control samples. Alagebrium was used as the reference compound[11].

Determination of dipeptidyl peptidase-4 activity *in vitro*. The inhibitory activity of the compounds towards dipeptidyl peptidase-4 was estimated as follows. Blood plasma (40 µL) was added to 50 µL of 0.1 M Tris-HCl buffer (pH 8,0). A solution of test compound (10 µL) with a specified concentration in Tris-buffer was added to the resulting mixture, and the mixture was pre-incubated at 37°C for 5 min. Then 100 µL of a 1 mM solution of Gly-Pro *p*-nitroanilide, the dipeptidyl peptidase-4 substrate (Sigma, USA), was introduced into the mixture. The reaction mixture was incubated at 37 °C for 15 min and the formation of *p*-nitroaniline was detected by measuring the absorbance at 405 nm using an Infinite M200 PRO microplate reader (Tecan, Austria)[12]. Vildagliptin (Sigma, USA) was used as the reference compound[13].

Determination of glycogen phosphorylase activity *in vitro*

The inhibitory activity of the compounds towards glycogen phosphorylase was determined as follows. 50 mM HEPES buffer (pH 7.2, 100 µL) containing 100 mM KCl, 2.5 mM $MgCl_2$, 0.5 mM glucose-1-phosphate (Sigma, USA), and 1 mg/mL glycogen was pre-incubated with rabbit muscle glycogen phosphorylase (Sigma, USA) and 5 µL of a solution of a test compound of a specified concentration at 30 °C for 30 min. Then 150 µL of a solution containing 1.05% $(NH_4)_2MoO_4$ and 0.034% malachite green was added to the reaction mixture. The mixture was incubated at 30 °C for 20 min, and the amount of the released phosphate anion was determined by measuring the absorbance at 620 nm using an Infinite M200 PRO microplate reader (Tecan, Austria)[14]. CP-316819 (Sigma, USA) was used as the reference compound[15].

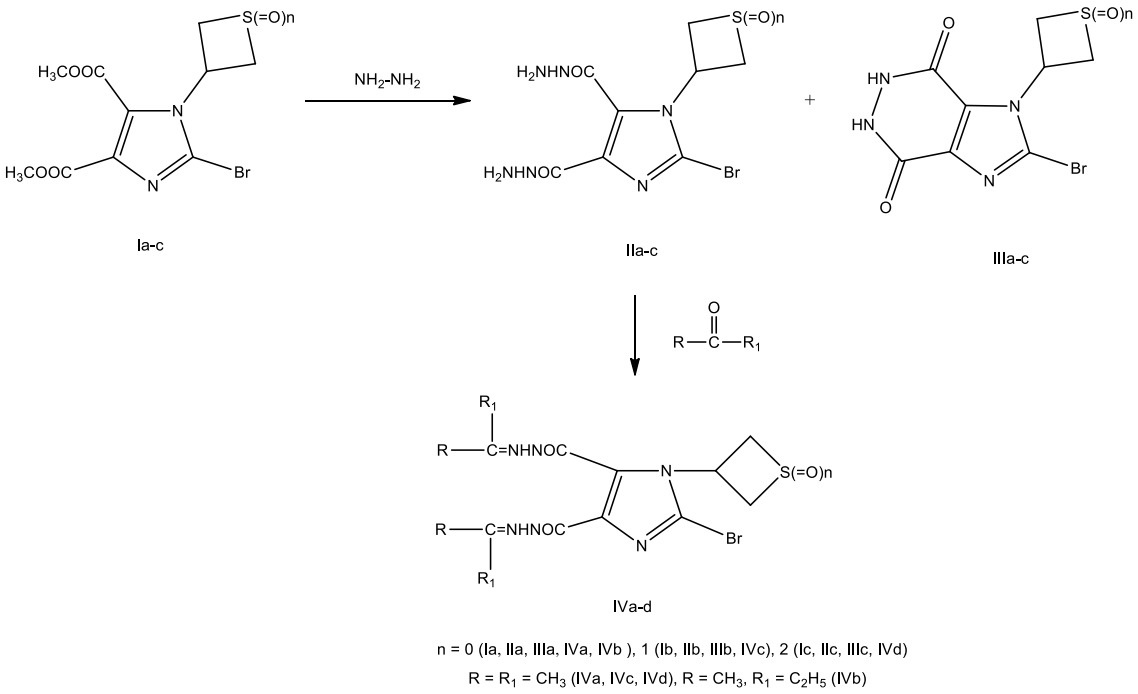
Determination of the α-glycosidase activity *in vitro*

The inhibitory activity of the compounds towards α-glycosidase was determined as follows. The test compounds in a specified concentration were pre-

incubated in 50 µL of 0.1 M phosphate buffer (pH 6.8) with 25 µL of a 5 mM solution of the substrate, 4-nitrophenyl-α-D-glucopyranoside, at 37 °C for 5 min. Then 25 µL of an α-glycosidase (*Saccharomyces cerevisiae*, Sigma, USA) solution, prepared by dissolving 1 mg of the enzyme in 100 mL of 0.1 M phosphate buffer (pH 6.8) containing 200 mg of bovine serum albumin, was added, and the mixture was incubated at 37 °C for 15 min. The absorbance increase at 400 nm was determined using an Infinite M200 PRO microplate reader (Tecan, Austria) [16]. Acarbose was used as the reference agent (Sigma, USA) [17].

RESULTS AND DISCUSSION

2-Bromo-1-(thietan-3-yl)-,2-bromo-1-(1-oxothietan-3-yl)-, 2-bromo-1-(1,1-dioxothietan-3-yl)imidazole-4,5-dicarbohydrazides (IIa-c) and 1-thietanyl-substituted 2-bromoimidazo[4,5-d]pyridazin-4,7(*5H,6H*)-diones (IIIa-c) were obtained by hydrazinolysis of dimethyl esters (Ia-c) by procedures reported in [7]. 2-Bromo-1-(thietan-3-yl)-, 2-bromo-1-(1-oxcothietan-3-yl)-,2-bromo-1-(1,1-dioxothietan-3-yl)imidazole-4,5-dicarboxylic acid di(methylethylidenehydrazides) (IVa, IVc, IVd) and 2-bromo-1-(thietan-3-yl)imidazole-4,5-dicarboxylic acid di(ethylethylidenehydrazide) (IVb) were prepared by condensation of 2-bromoimidazole-4,5-dicarbohydrazides (IIa-c) with acetone and methyl ethyl ketone (Scheme 1). The structures of compounds IVa-d were confirmed by ^{1}H NMR spectroscopy. Indeed, the ^{1}H NMR spectrum of IVa recorded in deuterated DMSO exhibited doubled signals as two singlets for the NH groups at 12.22 (*Z*), 11.18 (*E*) ppm and 10.42 (*Z*), 9.91 (*E*) ppm. The spectrum showed characteristic signals for the thietane ring, the NCH proton signals being at 6.23-6.31 (*Z*) and 5.59-5.63 (*E*) ppm and the signals for two $S(CH)_2$ groups occuring at 4.19-4.23 (*Z*), 4.05-4.09 (*E*) and 3.34-3.43 (*E*+*Z*) ppm. The proton signals for acetone residue are also doubled.



IVa-d

Scheme 1: Synthesis of the target compounds

The results of biological activity assays of the products are summarized in Table 1. Most of the compounds inhibit the formation of glycation end-products *in vitro*. The highest antiglycation activity was observed for dihydrazides IIa and IIc and diylidenhydrazides IVa. In the concentration range of 0.1-1 mM, they are comparable with aminoguanidine used as the reference. Considering the fact that bicyclic derivatives IIIa, IIIb, and IIIc in which the hydrazine moiety is rigidly bound are least active, we conclude that particularly the hydrazide group is crucial for the antiglycation properties. The mechanism of action of aminoguanidine, which can also be regarded as a hydrazine derivative, is based on the ability to bind reactive dicarbonyl compounds [18,19]. The hydrazides considered in the present communication are less nucleiohilic than aminoguanidine because of the negative mesomeric effect of the carbonyl group bound to the hydrazine residue. However, as described in [20], isoniazid, which is also a hydrazide, has a weak antioxidant activity but actively scavenges carbonyl compounds. Probably, the same mechanism of action is inherent in our glycation inhibitors IIa, IIc, and IVa. However, these compounds do not show a statistically significant deglycation activity.

Interesting results were obtained in a study of dipeptidyl peptidase-4 inhibition. The highest activity was found for compounds containing an oxothietane ring (IIb, IIIb, IVc). Compounds containing a

dioxothietane ring (IIc, IIIc, IVd) are somewhat less active as dipeptidyl peptidase-4 inhibitors. The lowest activity is characteristic for compounds containing non-oxidized thietane ring (IIa, IIIa, IVa, IVb). The structures of substituents in positions 4 and 5 are less significant. Nevertheless, it still can be concluded that hydrazide moieties or pyridazinedione ring, resulting from their cyclization, are most favorable for inhibition of dipeptidyl peptidase-4. Diylidenhydrazides were generally less active.

Experimental investigation of the compounds in question demonstrated that some of them have a statistically significant inhibitory activity towards glycogen phosphorylase. Dihydrazides IIa, IIb, and IVc and imidazo[4,5-*d*]pyridazine-4,7(5*H*,6*H*)-dione IIIb showed a moderate activity (22-38% inhibition). The most pronounced inhibitory properties (51%) were found for dihydrazide IIc containing a dioxothietane ring. However, this compound, too, is substantially inferior to the reference compound CP-316819.

Finally, it was found that imidazo[4,5-d]pyridazine-4,7(5*H*,6*H*)-dione IIIa is a marked α-glycosidase inhibitor. When present in 1 mM concentration, it is superior to the reference agent, acarbose, in the inhibition and is comparable with acarbose in the IC_{50} value. Other derivatives do not show significant activity of this type.

Table 1: The results of biological activity assays of the synthesized compounds

Compounds	Inhibition of the formation of glycation end-products		Deglycation activity		Dipeptidyl peptidase-4 inhibition	Glycogen phosphorylase inhibition	α-Glycosidase inhibition
	1×10^{-3} M	1×10^{-4} M	5×10^{-3} M	1×10^{-3} M	1×10^{-4} M	1×10^{-4} M	1×10^{-3} M
IIa	50.08±1.24*	6.70±1.67	-7.53±3.42	-8.13±12.93	28.21±4.47*	25.08±4.72*	-3.23±2.52
IIIa	25.29±1.37*	5.48±2.26	9.95±0.91	8.92±1.15	27.38±8.96	-94.16±0.31*	96.38±3.63* IC_{50} 660.6 µM
IVa	52.90±0.70*	15.36±4.36	10.93±0.61	8.59±0.81	11.10±4.88	-183.52±8.46*	10.92±2.13*
IVb	28.29±3.34*	4.02±2.27	9.82±1.03	9.21±0.81	25.73±8.27*	7.94±4.25	-5.98±9.52
IIb	24.57±1.69*	7.16±3.29	2.49±4.03	-3.87±1.62	63.76±7.72*	21.82±3.33*	-4.95±1.50
IIIb	17.90±4.68*	5.29±2.45	-0.96±2.84	1.83±1.35	51.31±2.80*	21.85±4.82*	-3.39±2.95
IVc	33.86±2.83*	19.01±4.22	6.28±1.98	12.26±0.05	49.03±5.11*	37.68±2.16*	0.52±0.52
IIc	46.51±2.90*	5.47±3.30	8.89±3.89	-2.53±2.11	43.79±7.92*	51.48±5.30*	10.34±2.42*
IIIc	0.71±3.46	-25.05±4.92*	3.28±2.92	-1.06±3.84	37.64±6.29*	1.08±7.95	1.92±1.33
IVd	15.21±1.90*	0.50±2.93	4.40±0.21	11.49±0.21	29.67±5.92*	-8.41±7.56	-10.54±5.67
Aminoguanidine	57.83±0.58*	6.01±2.12*	–	–	–	–	–
Alagebrium	–	–	34.18±2.01	22.86±0.98	–	–	–
Vildagliptin	–	–	–	–	92.93±1.81*	–	–
CP-316819	–	–	–	–	–	91.63±5.28*	–
Acarbose	–	–	–	–	–	–	67.73±2.49* IC_{50} 543.6 µM

*Statistically different from the negative control according to the Mann–Whitney U-test ($p<0.05$).

CONCLUSION

Thus, some of the synthesized 2-bromo-1-(thietan-3-yl)-, 2-bromo-1-(1-oxothietan-3-yl)-, and 2-bromo-1-(1,1-dioxothietan-3-yl)imidazole-4,5-dicarboxylic acid derivatives were found to exhibit various types of biological activity relevant to the therapy of type 2 diabetes mellitus. In particular, we have found compounds comparable with aminoguanidine in the antiglycation activity (IIa, IIc, and IVa). It was shown that imidazo[4,5-d]pyridazine-4,7(5*H*,6*H*)-dione IIIa is comparable in activity with the reference α-glycosidase inhibitor acarbose. Dihydrazide IIc exhibited both clear-cut anti-glycation properties and inhibitory properties towards dipeptidyl peptidase-4 and glycogen phosphorylase. Thus, this series of derivatives may be considered for further investigation to identify novel antidiabetic agents.

Acknowledgements: This work was supported by the Russian Science Foundation (Project No. 14-25-00139).

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Source of Support: Nil, **Conflict of Interest:** None.