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Dedicated to B.I. Buzykin on His 80th Anniversary

Synthesis and Antitubercular, Antimicrobial, and Hemolytic Activity of Methyl D-Glucopyranuronate and Its Simplest Derivatives

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Abstract—Methyl glucuronate and some of its simplest derivatives have been synthesized, and their antitubercular, antimicrobial, and hemolytic activities have been studied. The simplest derivatives of glucuronic acid have been shown for the first time to exhibit a high antitubercular activity which is comparable with the activity of isoniazid.

Keywords: glyucuronic acid, methyl D-glucopyranuronate, glucuronides, glucopyranosides, antitubercular activity

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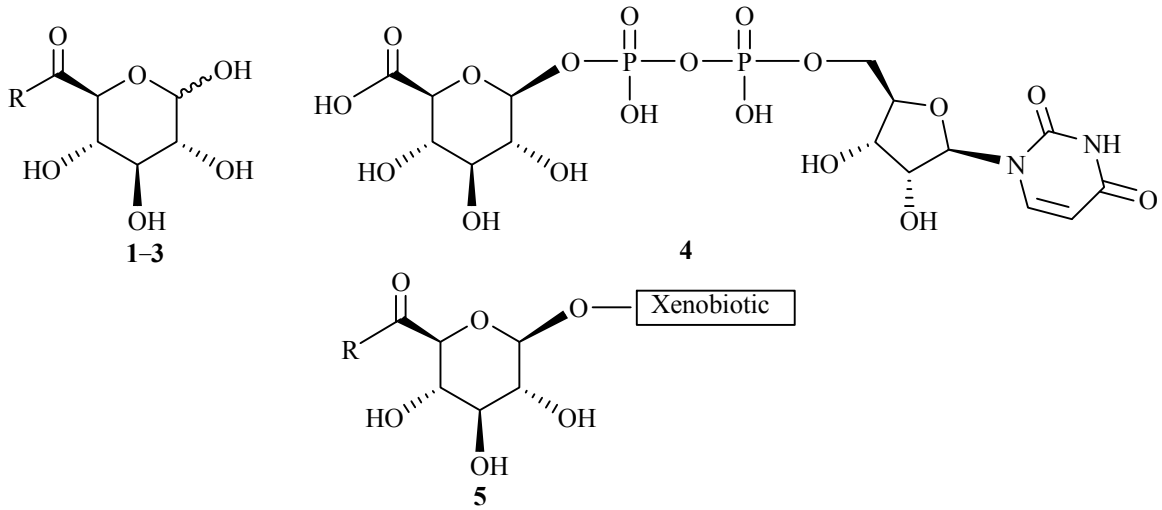
Glucuronic acid (**1**) is the most important participant of phase II metabolism of xenobiotics, including drugs, in human organism. Glucuronic acid is a structural fragment of uridine diphosphate glucuronic acid (**4**, UDPGA), which is a coenzyme ensuring UDP-glucuronosyl transferase-catalyzed glucuronidation of xenobiotics oxidized during phase I metabolism; water-soluble glucuronides **5** thus formed are excreted with

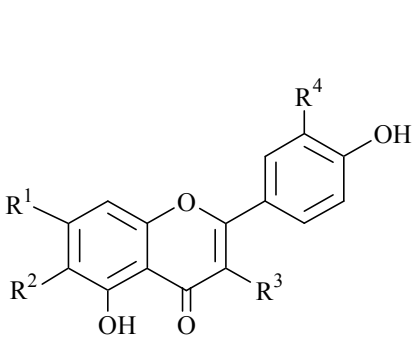
Scheme 1.



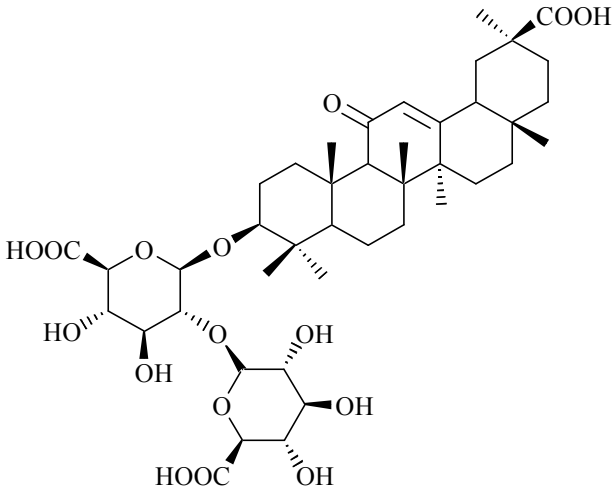
1–3 **4** **5**

R = OH (**1**), OCH_3 (**2**), NH_2 (**3**).

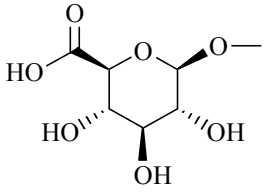
Scheme 2.

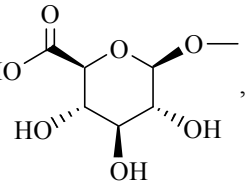


6–**8**



9

R^1 = OH, R^2 = H, R^3 = R^4 = OH (**6**); R^1 = OH, R^2 = H, R^3 =  R^4 = OH (**7**);

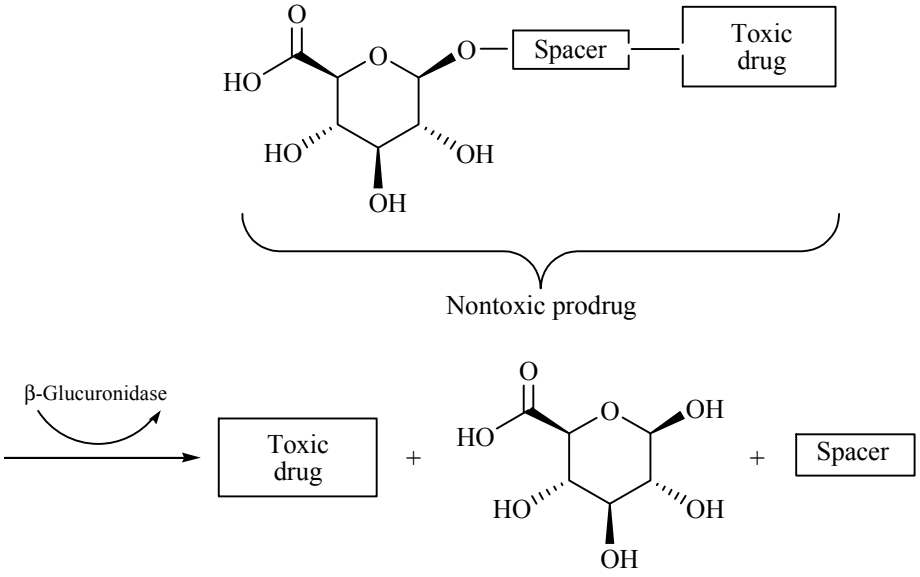
R^1 =  , R^2 = OH, R^3 = R^4 = H (**8**).

urine or are transported with blood from the liver to other organs [1, 2] (Scheme 1).

It should be noted that glucuronidation of xenobiotics taken by humans endows them with some pharmacological activity via transformation into beneficial compounds (glucuronides). For example, the natural flavonoid quercetin (**6**) widely known due to its biological activity (antioxidant, antitumor, anti-inflammatory, antidiabetic [3]), which occurs in vegetables, fruits, tea, and red wine [4], is metabolized in humans to quercetin 3-*O*-β-glucuronide (**7**), and the latter is transferred with blood to different tissues, including brain and muscles, where it performs its useful functions [3]. The major metabolite of quercetin, quercetin 3-*O*-β-glucuronide (**7**), responsible for its useful properties, is present as such in fruits, berries, and some vegetables (lettuce, green bean) [4]. This is not a single example. Metabolites (glucuronides) of the natural phenols tyrosol and hydroxytyrosol occurring in olives protect hemoglobin from oxidation and red blood cells from morphological change [5]. Many other falvonoid glucuronides isolated from higher plants display biological activity. For example, scutellarin **8** (4′,5,6-trihydroxyflavone-7-glucuronide) is a traditional Chinese medicine exhibiting cytotoxic activity against some human cancer cell lines [6, 7]; it is also used in clinical practice for the treatment of strokes [8]. Flavonoid glucuronides isolated from *L. Leucocephala* [9] and flowers of *Syzygium aromaticum* [10] showed cytotoxic effect against a number of cancer cell lines, while those isolated from *Scutellaria indica* possess anti-inflammatory activity [11] (Scheme 2).

Natural glucuronides derived from triterpenoids are also biologically active. The most known is glycyrrhizin (**9**), which is a carboxylic acid of the oleanane series containing two glucuronic acid residues); it was found to exhibit anti-inflammatory, antiulcer, antiviral, antitumor, and immunomodulatory activities [12–14]. Biological activities of tens of other triterpenoid glucuronides have also been reported (see, e.g., [15–18]). Therefore, glucuronidation is used to endow natural terpenoids with new biological properties [19–21].

Scheme 3.

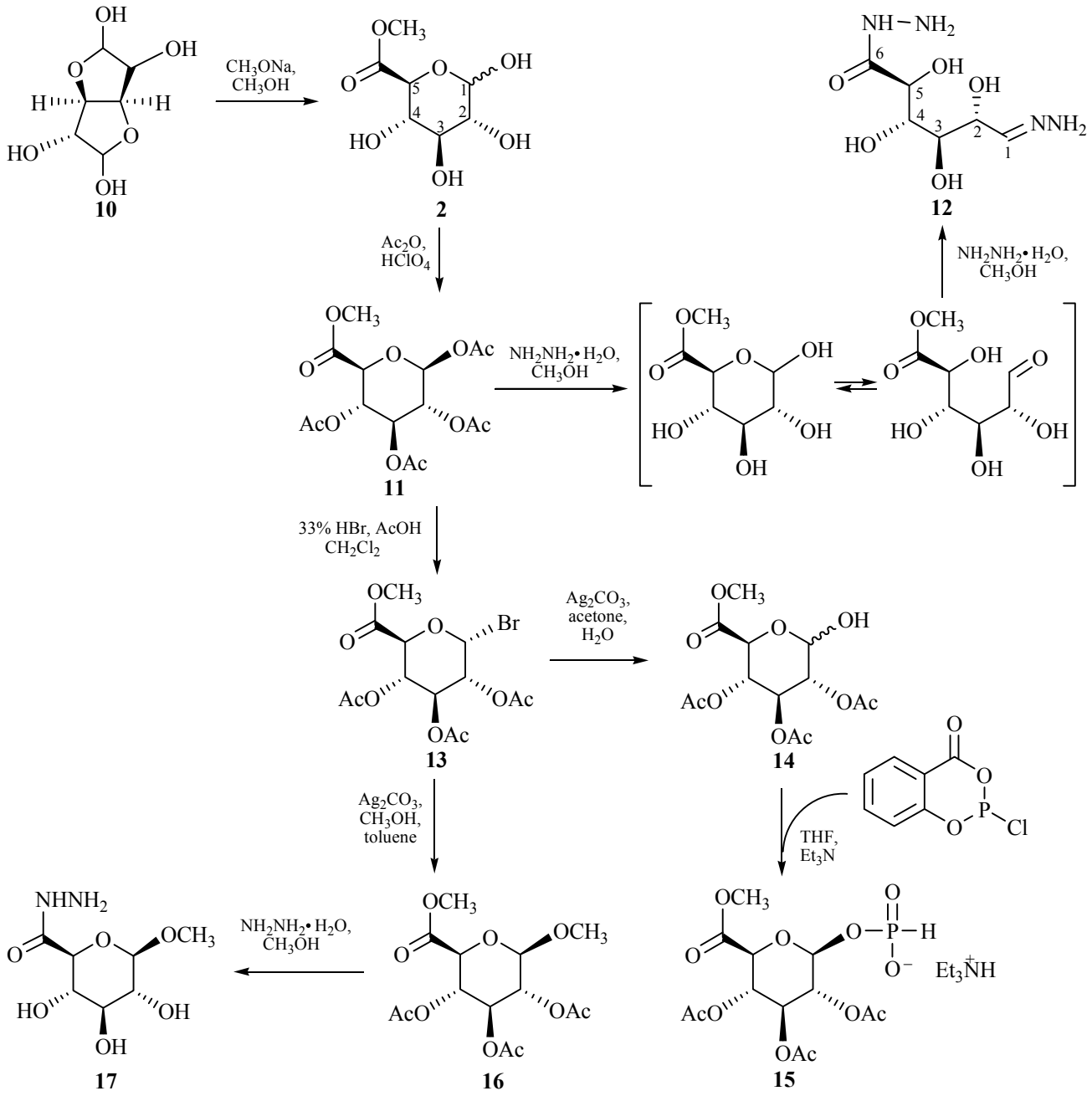


Glucuronic acid amide (**3**, glucuronamide) is a fragment of glycolipids in some bacteria [22]. Even more interesting is that amides of glucuronic acid and some amino acids, primarily L-alanine, constitute *O*-polysaccharides in a large number of bacteria, which act as antigens (see, e.g., [23–25]).

Taking into account that phase II metabolism of xenobiotics in human organism consists of their transformation into a water-soluble form for excretion, many therapeutic agents are subjected to glucuronidation to enhance their bioavailability or detoxification [26]. Detoxification of therapeutic agents as a result of their glucuronidation is widely used in the design drugs for chemotherapy of cancer [26–35]. In order to avoid damage to healthy cells, highly toxic anticancer agents (e.g., fluorouracil, anthracycline antibiotics, etc.) are subjected to glucuronidation [26, 27, 30, 31] or are covalently bound through a specific spacer to glucuronic acid [28, 32, 33], its methyl ester [28–30], or amide [34]. The resulting glucuronide conjugates (prodrugs) are not toxic, and they do not affect healthy cells. On the other hand, lysosomal β-glucuronidase present at a high concentration in cancer cells (especially in breast cancer cells [35]) hydrolyzes prodrugs to release cytotoxic anticancer agents directly at the target site (Scheme 3). Some prodrugs that are nontoxic due to the presence of glucuroric acid residues in their molecules include several cytotoxic anticancer agents [33]. After hydrolysis in cancer tissues by the action of β-glucuronidase, these prodrugs are converted to a highly toxic "cocktail" [33]. If a protein (usually, of the globulin series) is included in a prodrug, the latter becomes an immunogen [34]. Since β-glucuronidase recognizes as substrates only glucuronides (including prodrugs) with the glucopyranuronosyl residue containing free carboxy and hydroxy groups, glucuronic acid methyl ester **2** or even its 2,3,4-tri-*O*-acetyl derivative [27, 28, 36] was used as carbohydrate fragment instead of glucuronic acid **1** itself to avoid untimely hydrolysis of prodrugs in blood plasma. It was presumed [36] that it is only in cancer tissues that carboxylesterase removes acyl protection from the glucopyranuronate fragment of a prodrug and hydrolyzes its ester group; next follows hydrolysis of the prodrug by lysosomal β-glucuronidase, which releases the cytotoxic anticancer agent.

It should be noted that one of the pharmacophoric moieties in all biologically active natural (glucuronides derived from flavonoids [3–11], terpenoids [12–18], and polysachharides [23–25]) or synthetic glucuronides [19–21, 26–34, 36] listed above is glucuronic acid **1** [3–8, 11, 13–17, 26–28, 30–33], or its methyl ester **2** [9–11, 15, 16, 18, 19, 21, 26–30, 36], or amide **3** [19, 21, 23–25, 34], or even phosphite [21]. However, there are almost no published data on biological activity of these pharmacophores. It is only known that glucuronic acid hydroxamate exhibits antioxidant activity [37]. In order to fill the gap in the knowledge of biological activity of the above listed pharmacophores, in the present work we synthesized

Scheme 4.



glucuronic acid methyl ester **2** and a number of its simplest derivatives and studied their antitubercular, antimicrobial, and hemolytic activity.

Glucuronic acid methyl ester (**2**) was synthesized by treatment of D-(+)-glucurono-3,6-lactone **10** with sodium methoxide by analogy with the procedure described in [38]. The reaction was not stereoselective, and compound **2** was isolated as a mixture of α- and β-anomers (Scheme 4). The ^{1}H NMR spectrum of **2** contained a doublet at δ 5.38 ppm (^3J = 3.5 Hz) due to resonance of anomeric proton of the α-anomer and a doublet at δ 4.5 ppm (^3J = 10.0 Hz) corresponding to the β-anomer [39]. Acylation of **2** with acetic acid in the presence of perchloric acid gave tetra-*O*-acetyl derivative **11** which was isolated as pure β-anomer; the

Table 1. Antitubercular activity of compounds **2**, **11**, **12**, **15**, and **17** against *Mycobacterium tuberculosis* H37Rv

Comp. no.	MIC, μg/mL	Comp. no.	MIC, μg/mL
2	1.5	**15**	0.8
11	0.8	**17**	0.3
12	3.1	Isoniazid	0.1

anomeric proton of **11** resonated in the ^{1}H NMR spectrum as a doublet at δ 5.77 ppm (^3J = 7.7 Hz).

The reaction of **11** with hydrazine hydrate in methanol led to not only removal of the acetyl protecting groups but also hydrazinolysis of the ester group. Removal of the acetyl protection has made both ester and aldehyde (open-chain isomer) groups accessible for the reaction with hydrazine. As a result, hydrazono hydrazide **12** was obtained in 55% yield (Scheme 4). Compound **12** showed the molecular ion peak *m*/*z* 245.1 $[M + Na]^+$ in the ESI mass spectrum. Its IR spectrum contained absorption bands due to stretching vibrations of the hydrazide and hydrazone groups at 3300 (OH, NH_2), 1616 (amide I), 1535 (amide II), and 1349 cm^{-1} (amide III), as well as carbonyl stretching band at 1665 cm^{-1}. The ^{1}H NMR spectrum of **12** lacked signals assignable to acetyl and methoxy protons, and the anomeric proton (1-H) resonated as a doublet at δ 7.35 ppm (^3J = 6.2 Hz). The hydrazide NH proton signal was observed as a broadened singlet at δ 8.97 ppm, when the spectrum was recorded from a solution in DMSO-d_6. In the ^{13}C NMR spectrum of **12**, the C^1 signal (hydrazone moiety) characteristically appeared at δ_C 147.06 ppm. Two set of signals were observed in the ^{13}C NMR spectrum of **12**, indicating the presence of two isomers. The given spectral data are consistent with those reported in [40, 41].

By reaction of **11** with 33% HBr in AcOH by analogy with [42] we obtained bromide **13**. The anomeric proton of **13** resonated in the ^{1}H NMR spectrum as a doublet at δ 6.64 ppm (^3J = 4.1 Hz), indicating that the α-anomer was formed. Following the procedure described in [43], bromide **13** was converted to methyl-2,3,4-tri-*O*-acetyl-D-glucopyranuronate **14** which was reacted with 2-chloro-1,3,2-benzodioxaphosphinin-4-one to produce phosphonate **15** in 64% yield (Scheme 4). The ^{1}H NMR spectrum of **14** displayed doublets at δ 5.53 (^3J = 3.6 Hz) and 4.80 ppm (^3J = 7.6 Hz) corresponding to the α- and β-anomers, respectively. Phosphonate **15** showed only one set of signals in the ^{1}H NMR spectrum, and the anomeric proton resonated at δ 5.77 ppm as a doublet of doublets with the coupling constants $^3J_{HH}$ = 9.1 Hz and $^3J_{HP}$ = 3.4 Hz. The P–H proton signal was observed at δ 6.87 ppm as a doublet with a direct P–H coupling constant of 640.4 Hz. The ^{31}P NMR spectrum of **15** contained one singlet at δ_P 0.90 ppm. These finfings indicated that phosphonate **15** was isolated as pure β-anomer.

By analogy with the procedure given in [44], The Koenigs–Knorr reaction of **13** with methanol in the presence of silver carbonate afforded glucopyranuronate **16** which was isolated as the β-anomer, as followed from the presence of a doublet signal of the anomeric proton at δ 4.48 ppm (^3J = 7.7 Hz) in the ^{1}H NMR spectrum. In the final stage, compound **16** was treated with hydrazine hydrate to obtain 73% of hydrazide **17**. The IR spectrum of **17** contained absorption bands typical of stretching vibrations of the hydrazide moiety, whereas no acetyl proton signals were present in the ^{1}H NMR spectrum. The anomeric proton of **17** appeared in the ^{1}H NMR spectrum as a doublet at δ 4.05 ppm with a vicinal coupling constant of 7.8 Hz, which indicated conservation of the β-orientation of the glycoside bond.

The synthesized compounds were tested for antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv. The results are given in Table 1. The antitubercular (tuberculostatic) effects of methyl tetraacetylglucuronate **11**, phosphonate **15**, and 1-*O*-methyl-β-D-glucopyranuronohydrazide **17** were comparable with the activity of the antitubercular drug isoniazid used as reference. The activity of methyl glucuronate **2** and 1-hydrazono-D-glucuronohydrazide **12** was an order of magnitude lower, but it nevertheless exceeded the activity of the second-line antitubercular drug pyrazinamide by a factor of 4–8 (MIC 12.5 μg/mL [45]).

We also examined hemolytic activity of the synthesized glucuronic acid derivatives (Table 2). Compounds **2**, **11**, **12**, **15**, and **17** caused almost no damage to human erythrocytes (their hemolysis did not exceed 0.5%) at a concentration at which they showed antitubercular activity. Moreover, these compounds exhibited no hemolytic activity at concentrations 1000 times higher than their minimum inhibitory concentrations with respect to *Mycobacterium tuberculosis* H37Rv.

Furthermore, the synthesized compounds were tested for antimicrobial activity against gram positive

Staphylococcus aureus ATCC 209p and *Bacillus cereus* ATCC 8035, gram negative *Escherichia coli* CDCF-50 and *Pseudomonas aeruginosa* ATCC 9027, and fungi *Aspergillus niger* BKMF-1119, *Trichophyton mentagrophytes var. gypseum* 1773, and *Candida albicans* 855-653 (Table 3). Compound **12** showed a weak bacteriostatic activity against *S. aureus* and weak fungistatic activity against *C. albicans*. Compounds **2** and **11** also showed a weak activity against *C. albicans*, whereas hydrazide **17**, which was the most active antitubercular agent (Table 1), displayed no antimicrobial activity at all.

In summary, we were the first to demonstrate that the simplest glucuronic acid derivatives exhibit high antitubercular activity. Methyl 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranuronate **11**, triethylammonium (methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate) phosphonate **15**, and methyl β-D-glucopyranosiduronohydrazide **17** were are comparable in antitubercular activity to the first-line antitubercular drug isoniazid. The activity of methyl β-D-glucopyranuronate **2** and 1-hydrazono-D-glucuronohydrazide **12** exceeds the activity of the second-line antitubercular drug pyrazinamide (MIC 12.5 μg/mL) by a factor of 4–8.

EXPERIMENTAL

The IR spectra (400–4000 cm^{-1}) were recorded on a Bruker Vector 22 spectrometer (Germany) with Fourier transform; samples were examined as films or Nujol mulls. The ^{1}H, ^{13}C, and ^{31}P NMR spectra were measured on Bruker Avance-400 and Avance-600 spectrometers (Germany). The mass spectra (electrospray ionization) were obtained on a Bruker Daltonik AmazonX instrument (Bremen, Germany); positive ion detection, a.m.u. range 100–1500 Da, capillary voltage 4500 V, nebulizing gas nitrogen (200°C, flow rate 8 L/min); samples were dissolved in methanol or water to a concentration of 10^{-5} M; the data were processed using DataAnalysis 4.0 (Bruker). The optical rotations were

Table 2. Hemolytic activity of compounds **2**, **11**, **12**, **15**, and **17**

Comp. no.	Concentration, μg/mL	Hemolysis, %
2	100	0.3
	10	0
	1	0
	0.1	0
11	100	0.6
	10	0.3
	1	0
	0.1	0
12	100	0.3
	10	0.3
	1	0.2
	0.1	0
15	100	1.69
	10	0.5
	1	0.5
	0.1	0
17	100	0.5
	10	0.5
	1	0.5
	0.1	0.2

Table 3. Antimicrobial (bacteriostatic and fungistatic) activity of compounds **2**, **11**, **12**, and **17**

Compound no.	Minimum inhibitory concentration (MIC), μg/mL						
	Staphylococcus aureus	*Bacillus cereus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Aspergillus niger*	*Trichophyton mentagrophytes*	*Candida albicans*
2	>500	>500	>500	>500	>500	>500	250
11	>500	>500	>500	>500	>500	>500	250
12	250	>500	>500	>500	>500	>500	250
17	>500	>500	>500	>500	>500	>500	>500
Norfloxacine	2.4	7.8	1.5	3.0			
Ketoconazole	–	–	–	–	–	3.9	3.9

measured on a Perkin Elmer-341 polarimeter (USA). The progress of reactions and the purity of products were monitored by thin-layer chromatography on Sorbfil PTSKh-AF-A plates (Krasnodar, Russia); spots were visualized by treatment with a 5% solution of H_2SO_4 or 5% solution of $H_3PO_4 \cdot 12MoO_3 \cdot H_2O$, followed by heating. The products were isolated by column chromatography on silica gel (0.06–0.20 mm, Acros).

Methyl α/β-D-glucopyranuronate (2). Metallic sodium, 0.057 g (2.48 mmol), was dissolved in 30 mL of anhydrous methanol, and the solution was added to a solution of 5 g (28.4 mmol) of D-(+)-glucurono-3,6-lactone **10** in 20 mL of anhydrous methanol. The mixture was stirred for 5 h at room temperature, the solvent was removed under reduced pressure, and the residue was subjected to dry column chromatography using chloroform–methanol (5 : 1 to 3 : 1). Yield 2.91 g (49%), light yellow oil, $[\alpha]_D^{20}$ = 42.8° (*c* = 1.0, CH_3OH). ^{1}H NMR spectrum (600 MHz, D_2O), δ, ppm: 3.38–3.45 m (1H, 2β-H), 3.61–3.74 m (5H, 2α-H, 3α-H, 3β-H, 4α-H, 4β-H), 3.83–3.88 m (1H, 5α-H), 3.93 s (3H, CH_3O), 3.93 s (3H, CH_3O), 4.18 d (1H, 5β-H, *J* = 10.0 Hz), 4.50 d (1H, 1β-H, *J* = 10.0 Hz), 5.38 d (1H, 1α-H, *J* = 3.5 Hz). ^{13}C NMR spectrum (100 MHz, D_2O), δ_C, ppm: 55.60 and 55.63 (OCH_3), 73.23, 73.59, 73.79, 73.99, 74.84, 76.23, 77.19, 77.72 ($C^{2\alpha}$, $C^{2\beta}$, $C^{3\alpha}$, $C^{3\beta}$, $C^{4\alpha}$, $C^{4\beta}$, $C^{5\alpha}$, $C^{5\beta}$), 94.98 ($C^{1\alpha}$), 98.74 ($C^{1\beta}$), 173.55 and 174.50 (C=O). Mass spectrum (ESI), *m*/*z*: 231.1 $[M + Na]^+$, 247.0 $[M + K]^+$. Found, %: C 40.01; H 5.92. $C_7H_{12}O_7$. Calculated, %: C 40.39; H 5.81.

Methyl 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranuronate (11) was synthesized according to the procedure described in [38]. Yield 32%, mp 177°C (from MeOH), $[\alpha]_D^{20}$ = 8.1° (*c* = 1.1, $CHCl_3$); published data: mp 176°C [38], 177–178°C (from EtOH) [46], $[\alpha]_D^{25}$ = 7.4° (*c* = 2.0, $CHCl_3$) [46].

(2*S*,3*S*,4*R*,5*R*)-6-Hydrazinylidene-2,3,4,5-tetrahydroxyhexanohydrazide (12). Hydrazine hydrate, 4 mL (80.0 mmol), was added to a solution of 0.6 g (1.6 mmol) of glucopyranuronate **11** in 20 mL of anhydrous methanol. The mixture was kept for 24 h at room temperature, the precipitate was filtered off, and the solvent and excess hydrazine hydrate were removed under reduced pressure. Yield 0.18 g (54%), $[\alpha]_D^{20}$ = –8.7° (*c* = 0.5, H_2O). IR spectrum, ν, cm^{-1}: 3300 (OH, NH_2), 1665 (C=O), 1616 (amide I), 1535 (amide II), 1349 (amide III), 1080 (OH). ^{1}H NMR spectrum (600 MHz, D_2O), δ, ppm: 3.40–3.45 m, 3.58–3.70 m, 3.87–3.99 m, 4.21 d (*J* = 9.1 Hz), 4.31 d (*J* = 7.1 Hz), 4.37–4.41 m, 7.35 d (1H, 1-H, *J* = 6.2 Hz). ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm: 3.07–4.69 m, 6.91 d (1H, 1-H, *J* = 6.7 Hz), 8.97 br.s (1H, NH). ^{13}C NMR spectrum (100 MHz, D_2O), δ_C, ppm: 70.47, 70.76, 71.16, 71.25, 71.34, 71.80, 75.42, 76.19, 91.70, 147.06 (C=N), 169.54, 172.79 (C=O). Mass spectrum (ESI): *m*/*z* 245.1 $[M + Na]^+$. Found, %: C 32.67; H 6.73; N 25.38. $C_6H_{14}N_4O_5$. Calculated, %: C 32.43; H 6.35; N 25.21.

Methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate bromide (13) was synthesized as described in [42]. Yield 94%, mp 105–106°C (from EtOH); published data [46]: mp 106–107°C (from EtOH).

Methyl 2,3,4-tri-*O*-acetyl-α/β-D-glucopyranuronate (14) was synthesized as described in [43]. Yield 91%, mp 83°C; published data [43]: mp 81°C.

Triethylammonium (methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate) phosphonate (15). Glucopyranuronate **14**, 1 g (3.0 mmol), was dissolved in 8 mL of anhydrous THF, and 3 mL of anhydrous triethylamine and 0.67 g (3.3 mmol) of 2-chloro-1,3,2-benzodioxaphosphinin-4-one were added. The mixture was stirred for 24 h at room temperature, treated with 3 mL of water, and left to stand for 1 h. The solvent was removed under reduced pressure, and the product was isolated by column chromatography on silica gel (CH_2Cl_2–MeOH–Et_3N, 50 : 1 : 0.5 to 50 : 2 : 0.5). Yield 0.95 g (64%), light yellow oil, $[\alpha]_D^{20}$ = 33.5° (*c* = 1.0, $CHCl_3$). ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ, ppm: 1.30 t [9H, $N(CH_2C\underline{H}_3)_3$, *J* = 7.4 Hz], 1.93 s (3H, CH_3CO), 1.94 s (3H, CH_3CO), 1.96 s (3H, CH_3CO), 3.02 q [6H, $N(C\underline{H}_2CH_3)_3$, *J* = 7.5 Hz], 3.63 s (3H, CH_3O), 4.55 d (1H, 5-H, *J* = 10.2 Hz), 4.89–4.94 m (1H, 2-H), 5.09 t (1H, 4-H, *J* = 9.6 Hz), 5.52 t (1H, 3-H, *J* = 9.7 Hz), 5.77 d.d (1H, 1-H, *J* = 9.1, 3.4 Hz), 6.87 d (1H, PH, *J* = 640.4 Hz). ^{13}C NMR spectrum (100 MHz, $CDCl_3$), δ_C, ppm: 8.41 $[N(CH_2\underline{C}H_3)_3]$, 20.27 ($CH_3CO$), 20.43 (2C, CH_3CO), 45.64 $[N(\underline{C}H_2CH_3)_3]$, 52.48 ($CH_3O$), 68.89 ($C^3$), 69.08 ($C^5$), 69.42 ($C^4$), 70.07 d ($C^2$, J_{CP} = 5.5 Hz), 90.79 d (C^1, J_{CP} = 4.8 Hz), 167.92 ($\underline{C}OOCH_3$), 169.37 ($CH_3\underline{C}O$), 169.66 (2C, $CH_3\underline{C}O$). ^{31}P NMR spectrum (100 MHz, $CDCl_3$): δ_P 0.90 ppm. Mass spectrum (ESI): *m*/*z* 397.1 (I_{rel} 100%) $[M]^-$. Found, %: C 45.98; H 6.93; N 2.88; P 6.29. $C_{19}H_{34}NO_{12}P$. Calculated, %: C 45.69; H 6.86; N 2.80; P 6.20.

Methyl (methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosid)uronate (16) was synthesized as described in [44]. Yield 75%, mp 154–155°C (from EtOH); published data [44]: mp 154.1–154.5°C (from *i*-PrOH).

Methyl β-D-glucopyranosiduronohydrazide (17). Hydrazine hydrate, 3 mL (64.6 mmol), was added to a solution of 0.45 g (1.3 mmol) of glucopyranuronate **16** in 25 mL of anhydrous methanol, and the mixture was stirred for 30 min at room temperature. The precipitate was filtered off, washed with methanol, and dried under reduced pressure. Yield 0.21 g (73%), white powder, mp 233–234°C, $[\alpha]_D^{20}$ = –45° (*c* = 0.5, H_2O). IR spectrum, ν, cm^{-1}: 3417, 3350, 3332, 3284 (OH, NH, NH_2), 1672 (C=O), 1670 (amide I), 1557 (amide II), 1370 (amide III), 1029 (OH). ^1H NMR spectrum (600 MHz, DMSO-d_6), δ, ppm: 2.95–3.02 m (1H, 2-H), 3.10–3.17 m (1H, 3-H), 3.17–3.29 m (1H, 4-H), 3.30 s (3H, CH_3O), 3.38–3.42 m (1H, 5-H), 4.05 d (1H, 1-H, *J* = 7.8 Hz), 4.26 br.s (2H, NH_2), 4.95–5.16 m (3H, OH), 9.21 br.s (1H, NH). ^{13}C NMR spectrum (100 MHz, D_2O), δ_C, ppm: 57.47 (OCH_3), 71.20, 72.76, 74.45, 75.33, (C^2, C^3, C^4, C^5), 103.55 (C^1), 169.01 (C=O). Mass spectrum (ESI), *m*/*z*: 245.2 [*M* + Na]$^+$. Found, %: C 37.98; H 6.52; N 12.87. $C_7H_{14}N_2O_6$. Calculated, %: C 37.84; H 6.35; N 12.61.

Antitubercular activity. Compounds **2**, **11**, **12**, **15**, and **17** were tested for antitubercular activity against *Mycobacterium tuberculosis* H37Rv laboratory strain (hereinafter MBT) by the vertical diffusion method on a *Novaya* solid nutrient medium. The nutrient medium was placed in 5-mL test tubes and inoculated with an MBT suspension (0.1 mL) diluted to a turbidity value of 10 *GKI* units, and the test tubes were incubated for 24 h. The test tubes were then set in a vertical position, and 0.3 mL of a solution of compounds to be tested in aqueous ethanol at concentrations of 12.5, 6.2, 3.1, 1.5, 0.8, 0.3, and 0.1 μg/mL was added on the free edge of the tube. The test tubes were than placed in a thermostat and incubated for 10 days at 37°C under sterile conditions. The growth of MBT was assessed by the standard procedure according to which bacterial growth inhibition by 10 mm and more indicated tuberculostatic activity. The MBT growth inhibition zone (mm) is proportional to the tuberculostatic activity. A growth inhibition zone of 100 mm and more was assumed as complete inhibition. The antitubercular drug isoniazid was used as reference; it inhibited the growth of MBT with a MIC value of 0.1 μg/mL.

Antimicrobial activity. Compounds **2**, **11**, **12**, and **17** were tested for antimicrobial activity by the serial dilution method in liquid nutrient media according to [47]. The minimum inhibitory concentration (MIC) was determined, i.e., the concentration ensuring inhibition of growth and proliferation of test cultures. The following test cultures were used: gram positive: *Staphylococcus aureus* ATCC 209p, *Bacillus cereus* ATCC 8035; gram positive *Escherichia coli* CDCF-50, *Pseudomonas aeruginosa* ATCC 9027; funfi: *Aspergillus niger* BKMF-1119, *Trichophyton mentagrophytes var. gypseum* 1773, *Candida albicans* 855-653. Norfloxacin and ketoconazole were used as reference drugs.

Hemolytic activity. Compounds **2**, **11**, **12**, and **17** were tested for hemolytic activity against human erythrocytes by comparing the optical density of solutions of these compounds and dispersed human erythrocytes in saline with the optical density of blood after 100% hemolysis according to the procedure described in [48]. In keeping with GOST R ISO 10993 4-99, a compound is considered cytotoxic with respect to human erythrocytes if the hemolysis percentage exceeds 2%.

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