ISSN 1068-1620, Russian Journal of Bioorganic Chemistry, 2011, Vol. 37, No. 4, pp. 483–491. © Pleiades Publishing, Ltd., 2011.
Original Russian Text © V.E. Kataev, I.Yu. Strobykina, O.V. Andreeva, B.F. Garifullin, R.R. Sharipova, V.F. Mironov, R.V. Chestnova, 2011, published in Bioorganicheskaya Khimiya, 2011, Vol. 37, No. 4, pp. 542–551.

Synthesis and Antituberculosis Activity of Derivatives of *Stevia rebaudiana* Glycoside Steviolbioside and Diterpenoid Isosteviol Containing Hydrazone, Hydrazide, and Pyridinoyl Moieties

V. E. Kataev[a, 1], I. Yu. Strobykina[a], O. V. Andreeva[a], B. F. Garifullin[a], R. R. Sharipova[a], V. F. Mironov[a], and R. V. Chestnova[b]

[a] *A.E. Arbuzov Institute of Organic and Physical Chemistry, Kazan Scientific Center, Russian Academy of Sciences, ul. Arbuzova 8, Kazan, 420088 Russia*

[b] *Prophylactic Clinical Centre of the Republic of Tatarstan, Ministry of Health of the Republic of Tatarstan, ul. P. Lumumba 20, Kazan, 420045, Russia*

Received June 15, 2010; in final form, September 15, 2010

Abstract—Conjugates of the antituberculosis drug isoniazid (isonicotinyl hydrazine) and isomeric hydrazides of nicotinic and α-picolinic acid with glycoside steviolbioside from the *Stevia rebaudiana* plant and the product of its acid hydrolysis, diterpenoid isosteviol, were synthesized. In addition, isosteviol hydrazide and hydrazone derivatives as well as conjugates containing two isosteviol moieties joined by a dihydrazide linker were obtained. The parental compounds and their synthetic derivatives were found to inhibit the in vitro growth of *Mycobacterium tuberculosis* ($H_{37}R_V$). The measured minimal concentrations of stevioside and steviolbioside, at which the growth of *M. tuberculosis* was inhibited by 100% (MIC), were 7.5 and 3.8 μg/ml, respectively. MIC values for steviolbioside and isosteviol conjugates with hydrazides of pyridine carbonic acid were within the ranges of 5–10 and 10–20 μg/ml, respectively. The maximal inhibitory effect against *M. tuberculosis* was shown by the isosteviol conjugates with adipic acid dihydrazide (MIC 1.7 and 3.1 μg/ml). Antituberculosis activities of the tested compounds were higher than the activity of antituberculosis drug Pyrizanamide (MIC 20 μg/ml) but lower than that of antituberculosis drug isoniazid (MIC 0.02–0.04 μg/ml).

Keywords: antituberculosis activity, glycosides, isoniazid, isosteviol, S. rebaudiana, stevioside, steviolbioside

DOI: 10.1134/S1068162011030095

By now nearly a hundred natural compounds belonging to different classes have been reported that inhibit *Mycobacterium tuberculosis* growth at MIC from 100 to 3 μg/ml. Among them there are phenols, quinones, coumarins [1–3], peptides [1, 2], alkaloids [1, 2, 4, 5], terpenoids [1, 2, 6–18], steroids [1, 2, 19], and glycosides [20–22]. The analysis of these data allows for two essential conclusions. First, diterpenoids [1, 2, 6–14] and triterpenoids [1, 2, 15–18] are most abundant among secondary metabolites isolated from plant sources. It is noteworthy that they do not have nitrogen atoms, whereas all known drugs for tuberculosis treatment (isoniazid, etambutol, pyrazinamide, rifampicin, streptomycin, capreomycin, kanamycin, and others) [23], as well as compounds under preclinical and clinical trials [24–26], are nitrogen-organic compounds. This may imply that, in this case, we are dealing with a novel inhibitory mechanism of *M. tuberculosis* growth and, therefore, design of new antituberculosis terpenoid-derived agents is topical.

Second, glycosides occupy the lowest position in the list of natural tuberculostatics. According to our data there are only a few of them. They are saponin isolated from *Colubrina retusa* (MIC 10 μg/ml) [20], glycosides derived from 11-hydroxyhexadecane carbonic acid isolated from a *Ipomoea tyrianthina* methanol extract (MIC 25 μg/ml) [21], and glycosides of imberbic acid isolated from *Combretum imberbe* (MIC 100–12.5 μg/ml) [22]. It is interesting that, according to the published data, the antituberculosis activity of glycosides is lower than that of their aglycones. Particularly, MIC of triterpenoid imberbic acid was 1.56 μg/ml, whereas those of its glycosides, 100 to 125 μg/ml [22]. The MIC value of a triterpenoid aegicerin was 3.1 μg/ml [16], whereas the activity of the glycoside from *Scrophularia cryptophila* bearing a structurally identical aglycone was considerably lower (MIC > 100 μg/ml) [27]. Similarly, steroids isolated from *Thalia*

[1] Corresponding author: phone: +7 (843) 231-9160; fax: +7 (843) 273-2253; e-mail: kataev@iopc.ru.

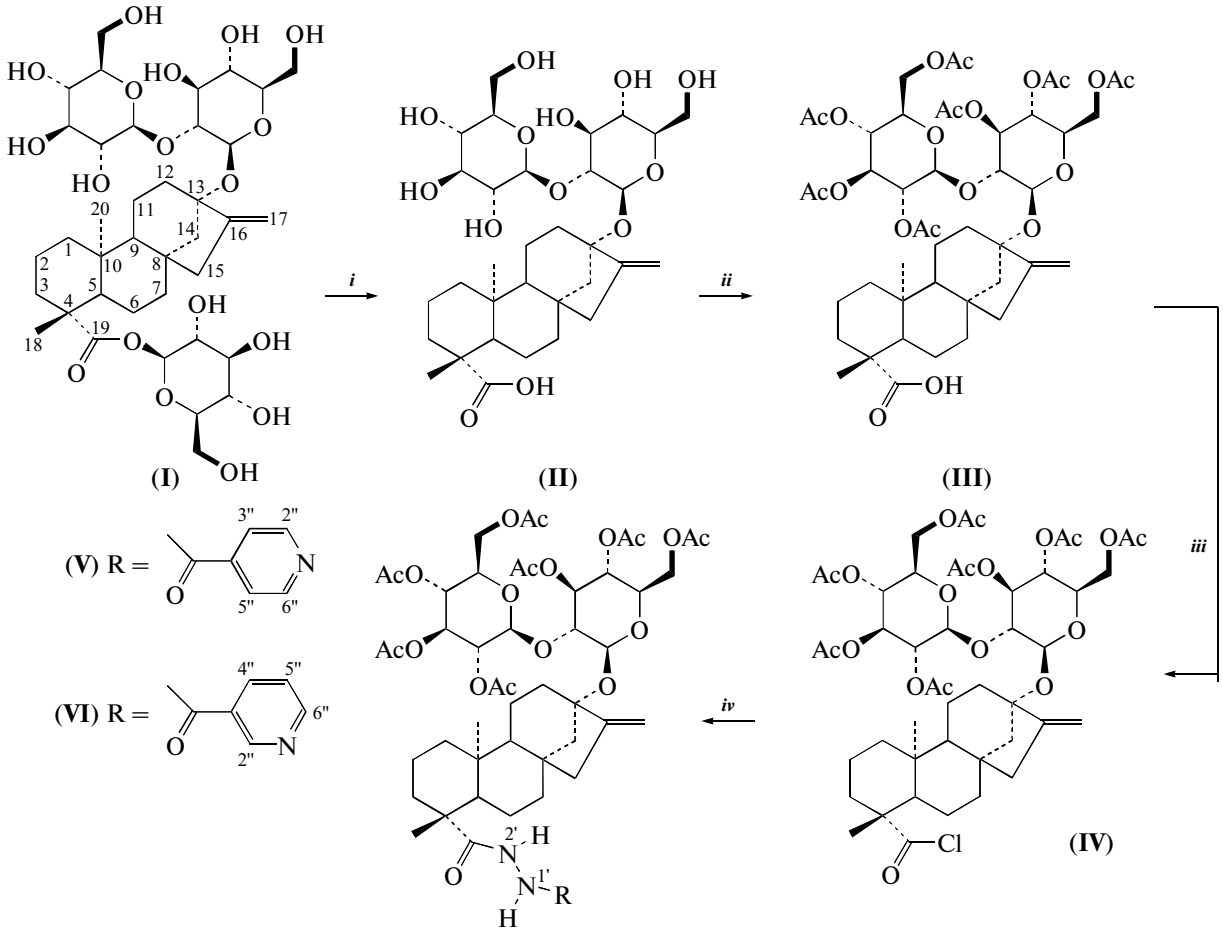
multiflora are much more toxic (MIC 1–4 μg/ml) than their glycosylated derivatives (MIC > 100 μg/ml) [19].

Considering the small number of published data on antituberculosis activity of natural glycosides, we studied the potential of stevibioside (**I**) and steviolbioside (**II**) to inhibit *M. tuberculosis* growth. These glycosides are 250 times sweeter than saccharose [28] and manifest antihypertensive [29, 30], antihyperglycemic, and insulinotropic [31–33] properties. In addition, taking into consideration the opinion described in [2] about uncommon antituberculosis agents among conjugates of metabolites displaying antituberculosis effects with synthetic mycostatics, another goal of this work was the synthesis of conjugates of steviolbioside (**II**) and the product of its acidic hydrolysis, diterpenoid isosteviol (16-oxo-*ent*-beyeran-19-oic acid) (**VII**) with an antituberculosis drug isoniazid (isonicotinyl hydrazine), as well as with isomeric hydrazides of other pyridine carbonic acids.

RESULTS AND DISCUSSION

For the synthesis of steviolbioside-based conjugates, we used the acid chloride approach. Hydrazides of isonicotinic and nicotinic acids were subjected to interaction with steviolbioside chloride (Scheme 1), in which sugar hydroxyl groups were preliminarily protected by acetyl groups to prevent their reaction with thionyl chloride.

Steviolbioside (**II**) was acetylated with acetic anhydride in pyridine under cooling [34]. In the IR spectrum of product (**III**), an absorption band at 1751 cm^{-1} corresponding to valence vibrations of AcO groups appeared. In the ^1H NMR spectrum of compound (**III**) protons, resonances of acetyl groups at 1.97–2.17 ppm arose in addition to resonances of steviolbioside (**II**) protons. Interaction of the resulting derivative (**III**) with an excess of $SOCl_2$ led to the corresponding acyl chloride (**IV**), which was immediately treated with hydrazides of pyridine carbonic acids (Scheme 1).



Reaction conditions: ***i*** NaOH (10%), 100°C, 1 h; ***ii*** Ac_2O, Py, 25°C; ***iii*** $SOCl_2$, 25°C; and ***iv*** $RNHNH_2$, Py/C_6H_6, 25°C.

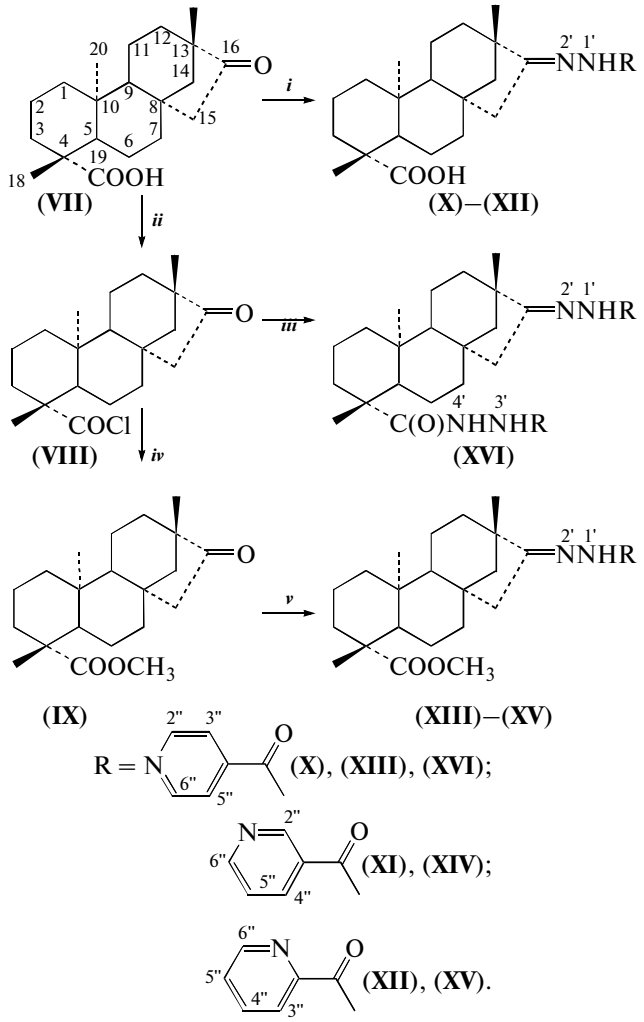
Scheme 1.

The reaction was carried out in a benzene–pyridine mixture at 20°C to give products (**V**) and (**VI**) in yields of 66 and 84%, respectively. In the IR spectra of these compounds, a band of valence vibrations of the stevi-

olbioside carboxylic group at 1690 cm^{-1} disappeared, whereas bands at 3300–3400 and 1590–1680 cm^{-1}, corresponding to valence vibrations of free NH groups and amide and aromatic fragments, respectively, appeared. The band at 1755 cm^{-1} supporting the presence of acetyl groups and the presence of pyridine rings in products (**V**) and (**VI**) was confirmed by the aromatic proton resonances at 7.83–8.80 and 7.40–8.75 ppm in the ^1H NMR spectra. In MALDI-TOF mass spectra, molecular peaks appeared at m/z 1056 $[M]^+$, 1078 $[M + Na]^+$, and 1094 $[M + K]^+$, corresponding to $C_{52}H_{69}N_3O_{20}$. It is noteworthy that an increase in the reaction temperature higher than 40°C led to partial hydrolysis of acetyl groups to give a mixture of chromatographically inseparable products, including exhaustively acetylated mixed hydrazide of steviolbioside and pyridine carbonic acid and hydrazides of the partially deacetylated compounds (normally primary hydroxyl groups are deprotected most smoothly).

It seemed interesting to synthesize similar conjugates derived from terpenoid isosteviol (**VII**), a product of acidic hydrolysis of *S. rebaudiana* glycosides [35], since it displayed not only hypotensive activity [36] but also an in vitro moderate tuberculostatic effect (MIC 50 μg/ml, strain $H_{37}R_V$) [14].

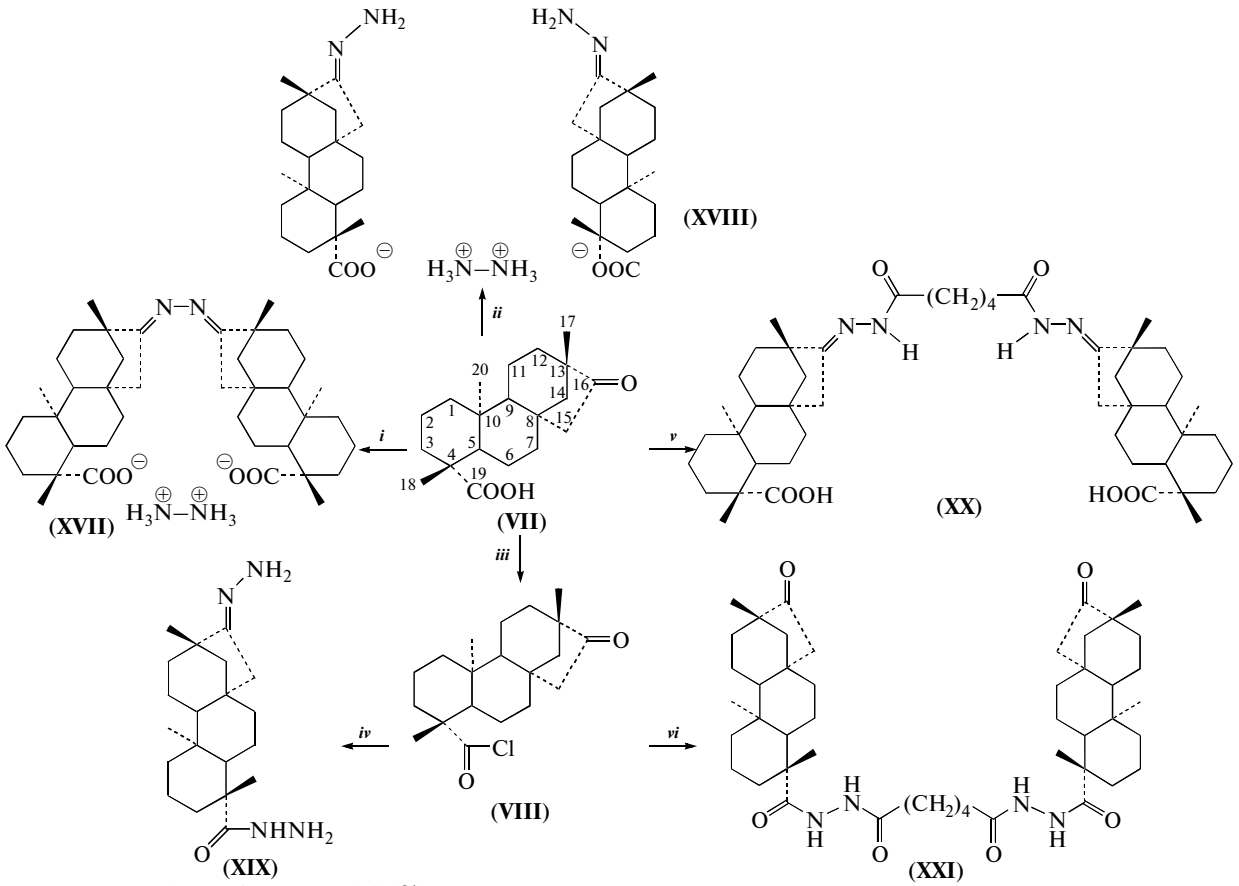
Reactions of isosteviol (**VII**) and its methyl ester (**IX**) with hydrazides of pyridine carbonic acids were carried out in anhydrous methanol in the presence of *p*-toluenesulfonic acid (*p*-TsOH) (Scheme 2). Conjugates (**X**)–(**XV**) were obtained in 75–85% yields. Isosteviol conjugate with two molecules of isoniazid (**XVI**) was synthesized in two steps. First, the reaction of isosteviol chloride (**VIII**) with an excess of isoniazid under heating in pyridine led to the product substituted at the acyl chloride residue. At the second stage, it reacted with an excess of isoniazid in boiling methanol in the presence of *p*-TsOH to give 55% of compound (**XVI**). The formation of derivatives (**X**)–(**XVI**) is confirmed by the disappearance in the IR spectrum of the band at 1740 cm^{-1} corresponding to valence vibrations of the isosteviol oxo group and appearance of bands of valence vibrations of C=N bonds and hydrazide groups (1540–1550, and 1620–1670 cm^{-1}) and the NH bond (3200–3500 cm^{-1}). ^1H NMR spectra of compounds (**X**)–(**XVI**) were similar for resonances of the isosteviol backbone of (0.7–3.0 ppm); resonances of pyridine protons were observed at 7.7–8.7 ppm; and the H1' resonance of the hydrazone residue, at 10.4–10.5 ppm. For compound (**XVI**) the resonances of hydrazone H1', H3' and H4' were seen at 10.43, 10.53, and 9.34 ppm, respectively.



Reaction conditions: *i* 1.5 mmol NH_2NH-R, CH_3OH (anhydrous), 0.1 mol *p*-TsOH, 65°C, 6 h; *ii* $SOCl_2$ (5-fold excess), 45°C, 1 h; *iii* 1 mol NH_2NH–R, C_6H_5N, 80°C, 12 h, then 0.3 mmol NH_2NH–R, CH_3OH (anhydrous), 0.1 mol *p*-TsOH, 65°C, 6 h; *iv* CH_3OH (anhydrous), 65°C, 1 h; and *v* 1.5 mmol NH_2NH–R, CH_3OH (anhydrous), 0.1 mol *p*-TsOH, 65°C, 6 h.

Scheme 2.

It was found earlier that covalent binding of two isosteviol (**VII**) molecules with a polymethylene spacer linked to the C16 atom allowed for the MIC reduction from 50 to 12.5 μg/ml [14], which corresponded to the activity of antituberculosis drug pyrazinamide (MIC 12.5–20 μg/ml) [23, 37, 38]. It was interesting to find the effect of pharmacophoric nitrogen-containing (hydrazide and hydrazone) fragments introduced in the spacer on the antituberculosis activity of the bisisosteviol derivatives. With this goal, we carried out reactions of diterpenoid isosteviol (**VII**) and its chloride (**VIII**) with hydrazine hydrate and adipic acid hydrazide (Scheme 3), and studied the potential of the products to inhibit *M. tuberculosis* growth.



Reaction conditions: ***i*** $N_2H_4 \cdot H_2O$ (98%), CH_3OH, 65°C, 8 h; ***ii*** N_2H_4, 60°C, 4 h; ***iii*** $SOCl_2$, 45°C, 1.5 h; ***iv*** N_2H_4, CCl_4, 60°C, 6 h; ***v*** $NH_2NHC(O)(CH_2)_4C(O)NHNH_2$, CH_3OH, 25°C, 12 h; ***vi*** $NH_2NHC(O)(CH_2)_4C(O)NHNH_2$, dioxane–pyridine, 50°C, 48 h.

Scheme 3.

Interaction of isosteviol (**VII**) with an excess of hydrazine hydrate in methanol yielded azine (**XVII**) isolated as a salt rather than isosteviol hydrazone (**XVIII**) (Scheme 3). Apparently, this can be explained by the reaction of the formed hydrazone (**XVIII**) with both starting isosteviol and the one resulted from the hydrazone (**XVIII**) hydrolysis. Homogeneous hydrazone (**XVIII**) was obtained by the reaction of isosteviol (**VII**) with a tenfold excess of anhydrous hydrazine.

Isosteviol hydrazide–hydrazone (**XIX**) was obtained by the interaction of isosteviol chloride (**VIII**) with anhydrous hydrazine in CCl_4. Its NMR spectrum contained singlets at 3.83 and 6.9 ppm corresponding to the resonance of hydrazide protons and a singlet at 4.75 ppm corresponding to the resonance of hydrazone protons along with characteristic resonances of isosteviol (**VII**) protons. It is noteworthy that although the synthesis of hydrazide–hydrazone (**XIX**) as well as that of hydrazone (**XVIII**) can be performed without a solvent in an excess of hydrazine, the use of an aprotonic hydrazine-immiscible solvent makes the reaction handier.

The reaction of isosteviol (**VII**) with adipic acid dihydrazide in methanol led to compound (**XX**), in which two isosteviol molecules are joined with a dihydrazide spacer at C16=O oxo groups (Scheme 3). In the ^{1}H NMR spectrum of bisderivative (**XX**), multiplet resonances at 1.5 and 2.0 ppm corresponding to the protons of adipic acid dihydrazide methylene group and a singlet at 10.1 ppm corresponding to the resonance of hydrazide protons were observed, along with characteristic resonances of isosteviol (**VII**). The reaction was carried out at room temperature, since heating led to hydrolysis of hydrazide–hydrazone (**XX**) followed by its transformation into azine (**XVII**) (Scheme 3).

The interaction of isosteviol chloride (**VIII**) with adipic acid dihydrazide in a dioxane–pyridine mixture resulted in compound (**XXI**), in which two isosteviol molecules are joined with a dihydrazide spacer at carboxylic C19OOH groups (Scheme 3). In the ^{1}H NMR spectrum of bisderivative (**XXI**), singlets at 8.91 and 9.46 ppm corresponding to the resonances of hydrazine protons as well as characteristic resonances of isosteviol (**VII**) were observed.

Biological tests of the compounds under study demonstrated that both starting glycosides (**I**) and (**II**) and terpenoid (**VII**) and their synthesized derivatives (**V**), (**VI**), and (**X**)–(**XXI**) displayed an in vitro moderate antituberculosis activity against *M. tuberculosis* ($H_{37}R_V$ strain). The MIC values for these compounds were within 1.7–50 μg/ml; the highest value (i.e., the worst case) (50 μg/ml [14]) was found for isosteviol diterpenoid (**VII**). As was proposed in [2], covalent binding of isosteviol (**VII**) to the antitubeculosis drug isoniazid and nicotinic and picolinic acid hydrazides allowed for a decrease in MIC values to 10–20 μg/ml from 50 μg/ml [14]. It is noteworthy that we did not observe a clear relationship between the antituberculosis activity and the nature and position of the hydrazide fragment in the isosteviol (*ent*-beyerane) backbone for conjugates (**X**)–(**XVI**): compounds (**X**), (**XII**), (**XIII**), and (**XVI**) inhibited *M. tuberculosis* growth at MIC 20 μg/ml, whereas compounds (**XI**), (**XIV**), and (**XV**), at 10 μg/ml.

As was mentioned above, according to limited data [16, 22, 27], the antituberculosis activity of glycosides [22, 27] was lower than that of the corresponding aglycones or structurally close compounds [22, 16]. On the contrary, we found that the activities of *S. rebaudiana* glycosides stevioside (**I**) (MIC 7.5 μg/ml) and steviolbioside (**II**) (MIC 3.8 μg/ml) were higher than that of isosteviol (**VII**) (MIC 50 μg/ml) [14]), which is isomeric to their aglycone steviol. It is also noteworthy that unlike isosteviol (**VII**), covalent binding of steviolbioside (**II**) to the antitubeculosis drug isoniazid and nicotinic and picolinic acid hydrazides did not result in an increase in its antituberculosis activity: MIC values for conjugates (**V**) and (**VI**) were 5 and 10 μg/ml, respectively.

We found that approximately tenfold augmentation of isosteviol (**VII**) antituberculosis activity if compared with its conjugates with hydrazides of pyridinic acids (**X**)–(**XVI**), including the one with isoniazid (**X**), (**XIII**), (**XVI**), could be achieved by its functionalization with hydrazide and/or hydrazone groups. The maximal activity in the range of the tested isosteviol derivatives was demonstrated by the compounds obtained by covalent binding of the two molecules to azine (**XVII**) or dihydrazide (**XX**) and (**XXI**) spacers. For example, MIC values for compounds (**XVII**–**XXI**) were 3.1, 6.3, 6.3, 3.1, and 1.6 μg/ml, respectively. One can assume that the growth of antituberculosis activity of the compounds bearing two isosteviol molecules linked with diester (MIC 12.5–25 μg/ml [14]), azine (MIC 3.1 μg/ml), and dihydrazide (MIC 1.6–3.1 μg/ml) spacers, as compared with isosteviol alone (MIC 50 μg/ml [14]), can be explained by an increase in hydrophobicity, which can facilitate penetration into the thick lipophilic cell wall of *M. tuberculosis*, as well as introduction of pharmacophoric nitrogen-containing groups. However, this is only a hypothesis, which requires further research.

To summarize, we first found the in vitro antituberculosis activity (strain $H_{37}R_V$) of *S. rebaudiana* glycosides stevioside (**I**) and steviolbioside (**II**), as well as synthesized conjugates of steviolbioside (**II**) and the product of its acidic hydrolysis, diterpenoid isosteviol (**VII**), with various hydrazides (**V**), (**VI**), and (**X**)–(**XXI**). The maximal inhibitory properties against *M. tuberculosis* were demonstrated by isosteviol conjugates with adipic acid dihydrazide (**XX**), (**XXI**), (MIC 3.1 and 1.6 μg/ml, respectively). The antituberculosis activity of the tested compounds exceeded that of the antituberculosis drug pyrazinamide (MIC 12.5–20 μg/ml [23, 27, 38]), but was lower than that of another drug isoniazid (MIC 0.02–0.04 μg/ml [38]). Indeed, the above described results are not sufficient for design of new semisynthetic antituberculosis agents based on structures of diterpenoids and their glycosides isolated from widely spread and regenerated plant sources. However, this work is the first step in this direction.

EXPERIMENTAL

IR spectra were recorded on a UR-20 spectrophotometer (Germany) in the range of 400–3600 cm^{-1} and on a Fourier Vector 22 spectrometer (Bruker, Germany) in the range of 400–4000 cm^{-1}. Crystalline samples were studied as suspensions in mineral oil. ^1H NMR spectra were registered on Avance-600 (Bruker, Germany, 600 MHz) and MSL-400 (Bruker, Germany, 400 MHz, δ, *J*, Hz) spectrometers. The melting points were measured on a Boetius microtable. Optical rotation was measured on a Perkin-Elmer M-341 (Germany) polarimeter. TLC analysis was carried out on Silufol UV-254 plates (Kavalier, Czech Republic). The compounds were developed by treatment with iodine. Column chromatography was performed on Silica gel 60 (0.06–0.2 mm, Alfa Aesar, England). Mass spectra in the mode of electron impact were recorded on an MX-1310 (Russia) spectrometer with an ionizing voltage of 60 eV, electron current of 30 μA, and a system of direct inlet into the ion source at 120°C. The ampoule–vaporizer was heated to 120–250°C. Accurate values of ion masses were determined by comparison with reference peaks of perfluorokerosene. MALDI mass spectra were registered on a MALDI-TOF ULTRAFLEX III mass spectrometer (Bruker, Germany). 2,5-Dihydroxybenzoic acid served as a matrix.

Isoniazid was purchased from Merck (Germany); nicotinic and picolinic acids were from Alfa Aesar (England).

Stevioside (**I**) was isolated from *S. rebaudiana* leaves [39]; steviolbioside (**II**) was obtained by alkaline hydrolysis of stevioside (**I**), similarly to [40]; pyridine carbonic acid hydrazides were synthesized as described in [41]; and isosteviol (**VII**) was obtained from a Sweta sweetener (Stevian Biotechnology Corp., Malaysia), as described in [42]. Isosteviol chlo-

ride (**VIII**) and methyl ester (**IX**) were synthesized using the method described in [43]. Physicochemical parameters of these compounds agreed with the published data.

13-*O*-[β-*D*-Heptaacetylsophorosyl]-*ent*-kauren-19-oic acid (III). Acetic anhydride (1.75 ml, 18 mmol) was added to a solution of steviolbioside (**II**) (0.5 g, 0.7 mmol) in anhydrous pyridine (5 ml) cooled to 0°C, and the mixture was kept at room temperature for 20 h. The mixture was heated at 60°C for 4 h and poured out into a cooled solution of 1% AcOH (40 ml). The precipitate was filtered off, washed with water, and dried on air to give 75% product (**III**) (0.55 g); mp 125°C (MeOH); $[\alpha]_D^{20}$ −39.5° (*c* 0.2, EtOH). IR (ν, cm^{-1}): 1754 ($OC(O)CH_3$), 1664 (C=C), 1230 (C–O). MS MALDI, *m/z*: 960 (937 + 23) ($M^+ + Na^+$). Found, %: C 58.80; H 6.94. $C_{46}H_{64}O_{20}$. Calc., %: C 58.95; H 6.89. ^1H NMR (C_5D_5N): 1.19 (3 H, s, H20), 1.34 (3 H, s, H18), 1.98,1.99, 2.01, 2.09, 2.1, 2.13, 2.17 (7 × 3 H, 7 s, Ac), 3.97–5.76 (14 H, m, H_{Sphr}), 5.05 (1H, s, H17), and 5.66 (1H, s, H17).

13-*O*-[β-*D*-Heptaacetylsophorosyl]-*ent*-kauren-19-oyl chloride (IV). Freshly distilled $SOCl_2$ (1 ml, 14 mmol) was added dropwise to heptaacetylated steviolbioside (**III**) (0.3 g) and the mixture was kept at room temperature for 24 h. The excess of $SOCl_2$ was evaporated and the residue was triturated with light petroleum and dried on air to give 95% product (**IV**) (0.29 g). IR (ν, cm^{-1}): 1814 (C1C=O), 1755 ($OC(O)CH_3$), 1230 (C–O).

The general procedure for preparation of hydrazides (V) and (VI). A solution of freshly prepared chloride (**IV**) (0.3 g, 0.3 mmol) in absolute benzene (3 ml, 33.7 mmol) was added dropwise to a solution of nicotinic or isonicotinic acid hydrazide (0.05 g, 0.3 mmol) in absolute pyridine (3 ml, 37.2 mmol), and the mixture was stirred at room temperature for 48 h. The solvent was evaporated and the residue was washed with water several times and recrystallized from methanol.

19-Nor-4α-(isonicotinoylhydrazinocarbonyl)-13-*O*-(β-*D*-sophorosyl)-*ent*-kauren (V). The yield was 0.22 g (66%); mp 140°C (MeOH); $[\alpha]_D^{20}$ −36.5° (*c* 0.5, MeOH). IR (ν, cm^{-1}): 3392, 3504 (NH), 1755 ($OC(O)CH_3$), 1645 (C=C, amide I), 1592, 1502 (Ar), 1232 (C–O). MS MALDI, *m/z* : 1056 (M^+), 1079 (1056 + 23) ($M^+ + Na^+$). Found, %: C 59.11; H 6.58; N 3.98. $C_{52}H_{69}N_3O_{20}$. Calc., %: C 59.12; H 6.6; N 3.98. ^1H NMR (DMSO-d_6): 0.96 (3 H, s, H20), 1.17 (3 H, s, H18), 1.55–2.08 (21 H, m, Ac), 3.94–5.24 (16 H, m, H_{Sphr}, H17), 7.83 (2 H, s, H3", H5"), 8.80 (2 H, br s, H2", H6"), 9.32 (1 H, s, H2'), 10.48 (1 H, s, H1').

19-Nor-4α-(nicotinoylhydrazinocarbonyl)-13-*O*-(β-*D*-sophorosyl)-*ent*-kauren (VI). The yield was 0.28 g (84%); mp 135°C (MeOH); $[\alpha]_D^{20}$ −33° (*c* 0.5, MeOH). IR (ν, cm^{-1}): 3469, 3371 (NH), 1754 ($OC(O)CH_3$), 1652 (C=C, amide I), 1592, 1502 (Ar), 1231 (C-O). MS MALDI, *m/z*: 960 (937 + 23) ($M^+ + Na^+$). Found, %: C 58.12, 58.96; H 6.46, 6.23; N 4.01, 4.04. $C_{52}H_{69}N_3O_{20}$. Calc., %: C 59.12; H 6.60; N 3.98. ^1H NMR ($CDCl_3$): 1.00 (3 H, s, H20), 1.31 (3 H, s, H18), 1.66–2.06 (21 H, m, Ac), 3.67–5.16 (16 H, m, H_{Sphr}, HI7), 7.39–7.41 (1 H, m, H5"), 8.18 (1 H, s, H4"), 8.21 (1 H, d, *J* 1.6, H6"), 8.75 (1 H, br s, H2"), 9.08 (1 H, s, H2'), 9.42(1 H, s, H1').

The general procedure for preparation of compounds (X)–(XV). Isosteviol (**VII**) or ester (**IX**) and pyridine carbonic acid hydrazide (1.5 eq) were dissolved in absolute methanol, a catalytic amount of p-TsCl was added, and the reaction mixture was refluxed for 6 h. Methanol was evaporated at reduced pressure. The residue was washed with water and recrystallized from methanol.

16-(4-Pyridinoylhydrazono)-*ent*-beyeran-19-oic acid (X). The yield was 0.23 g (85%); mp 315–328°C (MeOH). IR (ν, cm^{-1}): 1542 (amide II), 1650, 1664 (amide I), 1714 (C16=O), 3478, 3182 (NH). MS MALDI, *m/z*: 460 (437 + 23) ($M^+ + Na^+$). Found, %: C 70.58; H 8.01; N 9.07. $C_{26}H_{35}N_3O_3$. Calc., %: C 71.3; H 8.0; N 9.6. ^1H NMR (DMSO-d_6): 0.79 (3 H, s, H20), 1.08 (3 H, s, H17), 1.12 (3 H, s, H18), 2.02 (1 H, d, *J* 13.7, H3), 3.02 (1 H, dd, *J* 18.44,11.3, H15α), 7.68 (2 H, d, *J* 5.8, H2", H6"), 8.72 (2 H, d, *J* 5.8, H3", H5"), 10.50 (1 H, s, H1').

16-(3-Pyridinoylhydrazono)-*ent*-beyeran-19-oic acid (XI). The yield was 0.21 g (80%); mp 294–300°C (MeOH). IR (ν, cm^{-1}): 1547 (amide II), 1624 (amide I), 1718 (C16=O), 3055, 3219 (NH). MS MALDI, *m/z*: 438 (437 + 1) ($M^+ + H^+$). Found, %: C 71.59; H 9.01; N 9.60. $C_{26}H_{35}N_3O_3$. Calc., %: C 71.3; H 8.0; N 9.6. ^1H NMR (DMSO-d_6): 0.79 (3 H, s, H20), 1.08 (3 H, s, H17), 1.12 (3 H, s, H18), 2.02 (1 H, d, *J* 13.06, H3), 3.02 (1 H, dd, *J* 18.9, 2.3, H15α), 7.51 (1 H, m, H5"), 8.10 (1 H, m, H4"), 8.71 (1 H, m, H6"), 8.91 (1 H, s, H2"), 10.43 (1 H, s,H1').

16-(2-Pyridinoylhydrazono)-*ent*-beyeran-19-oic acid (XII). The yield was 0.18 g; (80%); mp 290–300°C (MeOH). IR (ν, cm^{-1}): 1515 (amide II), 1650, 1670 (amide I), 1710 (C16=O), 3338, 3106 (NH). MS MALDI, *m/z*: 460 (437 + 23) ($M^+ + Na^+$). Found, %: C 73.48; H 8.86; N 10.00. $C_{26}H_{35}N_3O_3$. Calc., %: C 71.3; H 8.0; N 9.6. ^1H NMR (DMSO-d_6): 0.79 (3 H, s, H20), 1.10 (3 H, s, H17), 1.13 (3 H, s, H18), 2.07 (1 H, dd, *J* 17.60, H3), 2.84 (1 H, dd, *J* 17.60, 2.25, H15α), 7.63 (1 H, m, H5"), 8.03 (1 H, m, H4"), 8.07 (1 H, m, H3"), 8.71 (1 H, d, *J* 4.5, H6"), 10.48 (1 H, s, H1').

Methyl 16-(4-pyridinoylhydrazono)-*ent*-beyeran-19-oate (XIII). The yield was 0.23 g (85%); mp 275–282°C (MeOH). IR (ν, cm^{-1}): 1552 (amide II), 1651, 1667 (amide I), 3200–3500 (NH). MS MALDI, *m/z*:

451 (M^+). Found, %: C 71.72; H 7.64; N 9.21. $C_{27}H_{37}N_3O_3$. Calc., %: C 72.0; H 8.0; N 9.0. ^{1}H NMR (DMSO-d_6): 0.67 (3H, s, H20), 1.08 (3 H, s, H17), 1.13 (3 H, s, H18), 2.05 (1 H, d, *J* 13.3, H3), 2.98 (1 H, dd, *J* 18.44, 1.3, H15α), 3.55 (3 H, s, H21), 7.68 (2 H, d, *J* 5.5, H2", H6"), 8.72 (2 H, d, *J* 5.5, H3", H5"), 10.48 (1 H, s, H1').

Methyl 16-(3-pyridinoylhydrazono)-*ent*-beyeran-19-oate (XIV). The yield was 0.18 g (75%); mp 245–249°C (MeOH). IR (ν, cm^{-1}): 1542 (amide II), 1638, 1653 (amide I), 1719 (C16=O), 3209, 3468 (NH). MS MALDI, *m/z*: 474 (451 + 23) (M^+ +Na$^+$). Found, %: C 71.8; H 7.7; N 9.01. $C_{27}H_{37}N_3O_3$. Calc., %: C 72.0; H 8.0; N 9.0. ^{1}H NMR (DMSO-d_6): 0.68 (3 H, s, H20), 1.05 (3 H, s, H17), 1.13 (3 H, s, H18), 2.05 (1 H, d, *J* 13.06, H3), 2.99 (1 H, dd, *J* 18.07, 2.1, H15α), 3.55 (3 H, s, H21), 7.52 (1 H, m, H5"), 8.10 (1 H, m, H4"), 8.72 (1 H, m, H6"), 8.92 (1 H, s, H2"), 10.40 (1 H, s, H1').

Methyl 16-(2-pyridinoylhydrazono)-*ent*-beyeran-19-oate (XV). The yield 0.16 g (60%); mp 185–190°C (MeOH). IR (ν, cm^{-1}): 1527 (amide II), 1653, 1692 (amide I), 1719 (C16=O), 3289, 3319 (NH). MS MALDI, *m/z*: 452 (451 + 1) (M^+ + H$^+$), 490 (451 + 39) (M^+ + K$^+$). Found, %: C 71.9; H 7.9; N 9.00. $C_{27}H_{37}N_3O_3$. Calc., %: C 72.0; H 8.0; N 9.0. ^{1}H NMR (DMSO-d_6): 0.67 (3 H, s, H20), 1.10(3 H, s, H17), 1.14 (3 H, s, H18), 2.82 (1 H, dd, *J* 5.8, 1.9, H15α), 3.58 (3 H, s, H21), 7.64 (1 H, m, H5"), 8.04 (2 H, m, H4", H3"), 8.70 (1 H, s, H6"), 10.48 (1 H, s, H1').

19-Nor-4α-(pyridinoylhydrazinocarbonyl)-16-(4-pyridinoylhydrazono)-*ent*-beyerane (XVI). 4-Pyridine carbonic acid hydrazide (0.15 g, 1.09 mmol) was added to a solution of isosteviol (**VII**) (0.18 g, 0.53 mmol) in anhydrous pyridine (10 ml, 0.126 mmol) and the reaction mixture was heated at a bath temperature of 80°C for 12 h. Pyridine was evaporated at reduced pressure and the residue was dissolved an anhydrous methanol. 4-Pyridine carbonic acid hydrazide (0.037 g, 0.27 mmol) and *p*-TsCl (0.02 g, 0.12 mmol) were added, and the mixture was refluxed for 6 h. Methanol was evaporated, and the residue was washed with water and recrystallized from ethanol to give 55% of the product (0.08 g); mp 189–192°C (EtOH). IR (ν, cm^{-1}): 1552 (amide II), 1651, 1667 (amide I), 3200–3500 (NH). MS MALDI, *m/z*: 556 (M^+). Found, %: C 65.10; H 7.62; N 13.64. $C_{32}H_{40}N_6O_3$. Calc., %: C 61.0; H 6.7; N 13.4. ^{1}H NMR (DMSO-d_6): 0.86 (3 H, s, H20), 1.08 (3 H, s, H17), 1.19 (3 H, s, H18), 2.23 (1 H, d, *J* 3.9, H3), 3.06 (1 H, dd, *J* 18.44,11.3, H15α), 7.67 (2 H, d, *J* 5.4, H2", H6"), 7.75 (2 H, d, *J* 5.4, H2", H6"), 8.70 (2 H, d, *J* 5.4, H3", H5"), 8.74 (2 H, d, *J* 5.4, H3", H5"), 9.34 (1 H, s, H4'), 10.43 (1 H, s, H1'), 10.53 (1 H, s, H3').

Hydrazinium 16,16'-azinodi(*ent*-beyeran-19-oate) (XVII). Hydrazine hydrate (0.63 ml, 12 mmol) was added to a solution of isosteviol (**VII**) (0.4 g, 1.2 mmol) in methanol (10 ml, 0.247 mmol). The mixture was refluxed for 6 h, methanol and an excess of hydrazine were evaporated, and the resulting precipitate was dried in vacuum with P_2O_5 to give 0.32 g (84%) of the product; mp 129–131°C (EtOH). IR (ν, cm^{-1}): 1182, 1264, 1661 (C=N). MS EI, *m/z*: 632.5 (M^+). Found, %: C 72.17; H 10.17; N 8.78. $C_{40}H_{64}N_4O_4$ ($C_{40}H_{60}N_2O_4 \cdot N_2H_4$). Calc., %: C 72.25; H 9.70; N 8.43. ^{1}H NMR ($CDCl_3$): 0.84 (3 H, s, H20), 1.06 (3 H, s, H17), 1.23 (3 H, s, H18), 2.16 (1 H, d, *J* 13.7, H3), 2.65 (1 H, dd, *J* 18.6, 3.7, H15α), 5.72 (3 H, s, N(+)$\underline{H}_3$).

Hydrazinium di(16-hydrazono-*ent*-beyeran-19-oate) (XVIII). Anhydrous hydrazine (0.3 ml, 10 mmol) was added to a solution of isosteviol (**VII**) (0.3 g, 1 mmol) in methanol (20 ml, 0.494 mmol). The mixture was stirred until a complete conversion of isosteviol (5 h), and methanol and unreacted hydrazine were removed. The residue was dried in vacuum with P_2O_5 to give 0.30 g (91%) of the product; mp 189–191°C (EtOH). IR (ν, cm^{-1}): 1167, 1453, 1662 (C=N), 3366 (NH_2). Found, %: C 68.02; H 9.70; N 12.61. $C_{40}H_{68}N_6O_4$ (2 $C_{20}H_{32}N_2O_4 \cdot N_2H_4$). Calc., %: C 68.93; H 9.83; N 12.06. ^{1}H NMR ($CDCl_3$): 0.85 (3 H, s, H20), 1.06 (3 H, s, H17), 1.23 (3 H, s, H18), 2.17 (1 H, d, *J* 13.7, H3), 2.66 (1 H,dd, *J* 18.6, 3.7, H15α), 3.91 (2 H, s, N$\underline{H}$).

19-Nor-4α-hydrazinocarbonyl-16-hydrazono-*ent*-beyerane (XIX). Thionul chloride (1 ml, 14 mmol) was added to isosteviol (**VII**) (0.36 g, 1.1. mmol) and the mixture was heated to 40°C until isosteviol (**VII**) was dissolved. An excess of $SOCl_2$ was removed in vacuum, and CCl_4 (5 ml, 51.8 mmol) was added to the dry residue. The mixture was stirred and the solvent was evaporated. The procedure was repeated until the smell of $SOCl_2$ disappeared. The dry residue was dissolved in CCl_4 (20 ml, 0.207 mmol) and anhydrous hydrazine (2 ml, 63 mmol) was added. The reaction mixture was refluxed for 4.5 h, and unreacted hydrazine was separated. The organic phase was evaporated to dryness, and the dry residue was dried in vacuum with P_2O_5 for 24 h to give 0.30 g (79%) of the product; mp 234–236°C (EtOH). IR (ν, cm^{-1}): 754, 982, 1451, 1629 (C=N), 3354 (NH_2). MS El, *m/z*: 346.3 (M^+). Found, %: C 68.12; H 9.86; N 16.19. $C_{20}H_{34}N_4O$. Calc., %: C 69.32; H 9.89; N 16.17. ^{1}H NMR ($CDCl_3$): 0.78 (3 H, s, H20), 1.05 (3 H, s, H17), 1.18(3 H, s, H18), 2.02 (1 H, d, *J* 13.7, H3), 2.63 (1 H, dd, *J* 18.6, 3.7, H15α), 3.83 (2 H, s, NH_2), 4.75 (2 H, s, N$\underline{H}_2$), 6.90 (1 H, s, N$\underline{H}$).

Adipic acid N^2,$N^{2'}$-bis(19-nor-4α-carboxy-*ent*-beyerasn-16-ylidene) dihydrazide (XX). Adipic acid dihydrazide (0.1 g, 0.6 mmol) was dissolved under heating in methanol (50 ml, 1.2 mmol), the solution was cooled, and isosteviol (**VII**) (0.38 g, 1.2 mmol) was added. The reaction mixture was stirred for 39 h, and the precipitate of unreacted adipic acid dihydrazide was filtered off. The filtrate was evaporated and the

Tuberculostatic activity in in vitro experiments of compounds (**I**)–(**III**), (**V**)–(**VII**), and (**X**)–(**XXI**)

Compound	MIC, μg/ml
(I)	7.5
(II)	3.8
(III)	5
(V)	5
(VI)	10
(VII)	50
(X)	20
(XI)	10
(XII)	20
(XIII)	20
(XIV)	10
(XV)	10
(XVI)	20
(XVII)	3.1
(XVIII)	6.3
(XIX)	6.3
(XX)	3.1
(XXI)	1.6
Pyrazinamide	20 [38]
Isoniazid	0.02–0.04 [38]

residue was stirred with chloroform (20 ml, 0.248 mmol). The mixture was filtered off, the filtrate was evaporated to dryness, and dried with P_2O_5 for 24 h to give 0.24 g (51%) of the product; mp > 300°C (EtOH). IR (ν, cm^{-1}): 753, 1125, 1453, 1635 (C=N), 1687 (COOH). MS MALDI, *m/z*: 775 (M^+). Found, %: C 71.00; H 9.55; N 7.18. $C_{46}H_{70}N_4O_6$. Calc., %: C 71.28; H 9.10; N 7.23. ^{1}H NMR ($CDCl_3$): 0.83 (6 H, s, H20), 1.14 (6 H, s, H17), 1.25 (6 H, s, H18), 1.53 (4 H, m, C<u>H</u>$_2$), 2.01 (4 H, m, C<u>H</u>$_2$), 2.33 (2 H, d, *J* 13.7, H3),2.91 (2 H, dd, *J* 18.6, 3.7, H15α), 10.05 (1 H, s, N<u>H</u>), 10.31 (1 H, s, N<u>H</u>).

Adipic acid N^2,$N^{2'}$-bis[(19-nor-16-oxo-*ent*-beyeran-4α-yl)carbonyl] dihydrazide (XXI). A solution of isosteviol chloride (**VIII**) (0.2 g, 0.6 mmol) in dioxane (3 ml) was added dropwise to a suspension of adipic acid dihydrazide (0.11 g, 0.5 mmol) in pyridine (10 ml), heated to 50°C in the argon atmosphere, and the reaction mixture was stirred at 50°C in the argon atmosphere for 24 h. Unreacted adipic acid dihydrazide was filtered off, the filtrate was evaporated in vacuum, and the residue was recrystallized from isopropanol to give 0.06 g (25%) of the product; mp 251–253°C (iso-PrOH). IR (ν, cm^{-1}): 1449, 1550 (amide II), 1659, 1737 (COOH), 3244. Found, %: C 71.78; H 8.84; N 6.58. $C_{46}H_{70}N_4O_6$. Calc., %: C 71.28; H 9.10; N 7.23. ^{1}H NMR ($CDCl_3$): 0.72 (6 H, s, H20), 0.87 (6 H, s, H17), 1.12 (6 H, s, H18), 2.13 (2 H, d, *J* 13.7, H3), 2.86 (2 H, dd, *J* 18.6, 3.7, H15α), 8.91 (2 H, s, N<u>H</u>), 9.46 (2 H, s, N<u>H</u>).

Antituberculosis activity of stevioside (**I**), steviolbioside (**II**), and compounds (**XVII**)–(**XXI**) was studied in vitro using the bacteriological method of vertical diffusion in the thick nutrient medium "Novaya." The laboratory strain $H_{37}R_V$ culture (10 mg) weighed on a torsion balance was placed in a porcelain mortar and carefully ground. Based on the bacterial standard, a culture suspension containing 100 million microbial particles per ml (10 U) was prepared. The resulting suspension (0.2 ml) was sown into tubes with the nutrient medium, and the tested solutions (0.3 ml) prepared by serial dilutions were added. The tubes were placed into a thermostat in a vertical position and incubated at 37°C for 10–12 days. The experiments were carried out in three repeats for each concentration. The MIC values are shown in the Table.

Antituberculosis activity of compounds (**V**), (**VI**), and (**X**)–(**XVI**) was studied on a BACTEC MGIT 960 system (United States). The Middlebrook 7H9 medium supplemented with BACTEC MGIT OADC (oleic acid, albumin, dextrose, and catalase) was used. Experiments were carried out by the method of serial dilutions on the *M. tuberculosis* $H_{37}R_V$ strain. The culture (10 mg) weighed on a torsion balance was placed in a porcelain mortar and carefully ground. Based on the bacterial standard, a culture suspension containing 100 million microbial particles per ml (10 U) was prepared. The resulting suspension (0.1 ml) was sown into tubes with the nutrient medium, and the tested solutions (0.5 ml) prepared by serial dilutions were added. The tubes were placed into a thermostat in a vertical position and incubated at 37°C. The growth of mycobacteria was registered daily for 11 days. The minimal inhibitory concentration of the synthesized compounds was determined as a minimal concentration, at which *M. tuberculosis* growth was delayed a day if compared with isoniazid. The MIC values are shown in the table.

ACKNOWLEDGMENTS

The authors are grateful to G.G. Mordovskii (The Urals Research Institute of Phthisiopulmonology, Ekaterinburg, Russia) for studies of antituberculosis activity of compounds (**I**), (**II**), (**XVII**)–(**XXI**); and R.Z. Musin and V.M. Babaev for registration of mass spectra. The work was supported by the program of Presidium of Russian Academy of Sciences no. 5, "Fundamental science to medicine," and the Russian Foundation for Basic Research, project no. 10-03-00499-a.

REFERENCES

1. Copp, B.R. and Pearce, A.N., *Nat. Prod. Rep.*, 2007, vol. 24, pp. 278–297.

2. Rogoza, L.N., Salakhutdinov, N.F., and Tolstikov, G.A., *Mini-Rev. Org. Chem.*, 2009, vol. 6, pp. 135–151.
3. Appendino, G., Mercalli, E., Fuzzati, N., Arnoldi, L., Stavri, M., Gibbons, S., Ballero, M., and Maxia, A., *J. Nat. Prod.*, 2004, vol. 67, pp. 2108–2110.
4. Kishore, N., Mishra, B.B., Tripathi, V., and Tiwari, V.K., *Fitoterapia*, 2009, vol. 80, pp. 149–163.
5. Rukachaisirikul, T., Innok, P., and Suksamrarn, A., *J. Nat. Prod.*, 2008, vol. 71, pp. 156–158.
6. Mongkolvisut, W. and Sutthivaiyakit, S., *J. Nat. Prod.*, 2007, vol. 70, pp. 1434–1438.
7. Hussein, A.A., Meyer, J.J.M., Jimeno, M.L., and Rodr?guez, B., *J. Nat. Prod.*, 2007, vol. 70, pp. 293–295.
8. Thongnest, S., Mahidol, C., Sutthivaiyakit, S., and Ruchirawat, S., *J. Nat. Prod.*, 2005, vol. 68, pp. 1632–1636.
9. Vik, A., James, A., and Gundersen, L., *Planta Med.*, 2007, vol. 73, pp. 1410–1412.
10. Kanokmedhakul, S., Kanokmedhakul, K., Kanarsa, T., and Buayairaksa, M., *J. Nat. Prod.*, 2005, vol. 68, pp. 183–188.
11. Gordien, A.Y., Gray, A.I., Franzblau, S.G., and Seidel, V., *J. Ethnopharmacol.*, 2009, vol. 126, pp. 500–505.
12. Thongtan, J., Kittakoop, P., Ruangrungsi, N., Saenboonrueng, J., and Thebtaranonth, Y., *J. Nat. Prod.*, 2003, vol. 66, pp. 868–870.
13. Molina-Salinas, G.M., Borquez, J., Ardiles, A., Said-Fernández, S., Loyola, L.A., San-Martin, A., Gonzalez-Collado, I., and Pena-Rodriguez, L.M., *Fitoterapia*, 2010, vol. 81, pp. 50–54.
14. Kataev, V.E., Militsina, O.I., Strobykina, I.Yu., Kovylyaeva, G.I., Musin, R.Z., Fedorova, O.V., Rusinov, G.L., Zueva, M.N., Mordovskoi, G.G., and Tolstikov, A.G., *Khim.-Farm. Zh.*, 2006, vol. 40, pp. 12–13.
15. Jimenez-Arellanes, A., Meckes, M., Torres, J., and Luna-Herrera, J., *J. Ethnopharmacol.,* 2007, vol. 111, pp. 202–205.
16. Rojas, R., Caviedes, L., Aponte, J.C., Vaisberg, A.J., Lewis, W.H., Lamas, G., Sarasara, C., Gilman, R.H., and Hammond, G.B., *J. Nat. Prod.*, 2006, vol. 69, pp. 845–846.
17. Pattamadilok, D. and Suttisri, R., *J. Nat. Prod.*, 2008, vol. 71, pp. 292–294.
18. Cantrell, C.L., Rajab, M.S., Franzblau, S.G., and Fischer, N.H., *J. Nat. Prod.*, 1999, vol. 62, pp. 546–548.
19. Gutierrez-Lugo, M., Wang, Y., Franzblau, S.G., Suarez, E., and Timmermann, B.N., *Phytother. Res.*, 2005, vol. 19, pp. 876–880.
20. ElSohly, H.N., Danner, S., Li, X.C., Nimrod, A.C., and Clark, A.M., *J. Nat. Prod.*, 1999, vol. 62, pp. 1341–1342.
21. Leon-Rivera, I., Miron-Lopez, G., Molina-Salinas, G.M., Herrera-Ruiz, M., Estrada-Soto, S., Gutierrez, M.C., Alonso-Cortes, D., Navarrete-Vazquez, G., Ros, S., and Said-Fernandez, M.Y., *J. Nat. Prod.*, 2008, vol. 71, pp. 1686–1691.
22. Katerere, D.R., Gray, A.I., Nash, R.J., and Waigh, R.D., *Phytochemistry*, 2003, vol. 63, pp. 81–88.
23. Bogadel'nikova, I.V. and Perel'man, M.I., *Antibakterial'naya terapiya tuberkuleza legkikh* (Antibacterial Therapy of Pulmonary Tuberculosis), Moscow: Universum Publishing, 1997.
24. Janin, Y.L., *Bioorg. Med. Chem.*, 2007, vol. 15, pp. 2479–2513.
25. Gutierrez-Lugo, M.-T. and Bewley, C.A., *J. Med. Chem.*, 2008, vol. 51, pp. 2606–2612.
26. Palomino, J.C. and Ramos, D.F., Almeida da Silva P., *Curr. Med. Chem.*, 2009, vol. 16, pp. 1898–1904.
27. Tasdemir, D., Brun, R., Franzblau, S.G., Sezgin, Y., and Calis, I., *Phytomedicine*, 2008, vol. 15, pp. 209–215.
28. Philips, K.C., in *Developments in Sweeteners*, vol. 3: *Stevia: Steps in Developing a New Sweetener*, Grenby, T.H., Ed., New York: Elsevier, 1987, pp. 1–43.
29. Chan, P., Tomlison, B., Yi-Jen, Ch., Ju-Chi, L., Hsieh, M.-H., and Cheng, J.-T., *Br. J. Clin. Pharmacol.*, 2000, vol. 50, pp. 215–220.
30. Hsieh, M.H., Chan, P., Sue, Y.M., Liu, J.C., Liang, T.H., Huang, T.Y., Tomlinson, B., Chow, M.S., Kao, P.F., and Chen, Y., *Clin. Therapeutics*, 2003, vol. 25, pp. 2797–2808.
31. Jeppesen, P.B., Gregersen, S., Rolfsen, S.E.D., Jepsen, M., Colombo, M., Agger, A., Xiao, J., Kruhoffer, M., Orntoft, T., and Hermansen, K., *Metabolism*, 2003, vol. 52, pp. 372–378.
32. Jeppesen, P.B., Gregersen, S., Alstrup, K.K., and Hermansen, K., *Phytomedicine*, 2002, vol. 9, pp. 9–14.
33. Gregersen, S., Jeppesen, P.B., Holst, J.J., and Hermansen, K., *Metabolism,* 2004, vol. 53, pp. 73–76.
34. Vis, E. and Fletcher, H.G., *J. Am. Chem. Soc.*, 1956, vol. 78, pp. 4709–4710.
35. Mosettig, E. and Nes, W.R., *J. Org. Chem.*, 1955, vol. 20, pp. 884–890.
36. Wong, K.L., Yang, H.Y., Chan, P., Cheng, T.H., Liu, J.C., Hsu, F.L., Liu, I.M., Cheng, Y.W., and Cheng, J.T., *Planta Med.*, 2004, vol. 70, pp. 108–112.
37. Cynamon, M.H., Speirs, R.J., and Welch, J.T., *Antimicrob. Agents Chemother.*, 1998, vol. 42, pp. 462–463.
38. Donald, P.R., *Tuberculosis*, 2010, vol. 90, pp. 279–292.
39. Kovylyaeva, G.I., Bakaleinik, G.A., Strobykina, I.Yu., Gubskaya, V.I., Sharipova, R.R., Al'fonsov, V.A., Kataev, V.E., and Tolstikov, A.G., *Khimiya Prirod. Soed.*, 2007, vol. 43, pp. 81–85.
40. Wood, H.B., Allerton, R., Diehl, H.W., and Fletcher, H.G., *J. Org. Chem.*, 1955, vol. 20, pp. 875–883.
41. Buu-Hoi, Ng.Ph., Xuong, Ng.D., Nam, Ng.H., Binon, F., and Royer, R., *J. Chem. Soc.*, 1953, pp. 1358–1364.
42. Khaibullin, R.N., Strobykina, I.Yu., Kataev, V.E., Lodochnikova, O.A., Gubaidullin, A.T., and Musin, R.Z., *Zh. Obshch. Khim.*, 2009, vol. 79, pp. 795–799.
43. Al'fonsov, V.A., Bakaleinik, G.A., Kataev, V.E., Kovylyaeva, G.I., Konovalov, A.I., Strobykina, I.Yu., Andreeva, O.V., and Korochkina, M.G., *Zh. Obshch. Khim.*, 2000, vol. 70, p. 1406.