INTRODUCTION

The problem of designing new medicines for treatment and preventive maintenance of viral infections and immunodeficiencies of various etiologies is one of the most important in medicine, pharmacy, and the medicinal chemistry because of the proceeding of the HIV infection distribution, viral hepatitides B and C, and herpetic infections. New viral respiratory infections are appearing such as Severe Acute Respiratory Syndrome (SARS), Avian Influenza, etc. Therefore, antiviral preparations are among the most intensively developed medicinal products in recent years. A prospective approach in the solution of the problem is the search for new antiviral agents among the natural compounds obtained from the available raw material of plant origin, their derivatives, and modified analogues.

GA (I), a main triterpene saponin in the extract of roots of licorice (Glycyrrhiza glabra L.) and Ural Glycyrrhyza uralensis Fisher are among the natural compounds that are of interest as base structures for the creation of new highly effective antiviral preparations and means for treatment and preventive maintenance of immunodeficiencies of various etiologies. These plants are widespread in the territory of Western Siberia, Southern Urals, Central Asia, Afghanistan, East China, Mongolia, Spain, and other regions [1]. GA and its monoammonium salt, Glycirram, completely inhibit in vitro the reproduction of some DNA and RNA viruses (Vaccinia, New Castle, Vesicular stomatitis, Herpes simplex, Herpes B, and Varicella zoster) [2]. GA is a leading natural glycoside suitable for prolonged therapy of the HIV infection and viral hepatitides B and C [3–5]. A unique property of GA is its ability to exhibit an anti-HIV effect already at early stages of the virus’s replication cycle and inhibit the stage of the virus’s adsorption by a cell [6].

A new class of triterpene glycopeptides in which the amide type of bonds intrinsic of natural glycoproteins is modeled [7, 8] is of a special interest among GA derivatives as immunomodulators. It has been earlier shown that some GA glycopeptide derivatives containing three amino acid residues or their methyl esters possess high immunotropic and anti-HIV-1 activity [7–12].

RESULTS AND DISCUSSION

We continue in this work a series of studies directed at the synthesis of new biologically active substances based on GA and related compounds.

Synthesis and Biological Activity of New Glycyrrhizic Acid Conjugates with Amino Acids and Dipeptides

L. A. Baltina, Jr., R. M. Kondratenko, L. A. Baltina, N. Z. Baschenko, and O. A. Pl'yasunova

Abstract—New glycyrrhizic acid (GA) conjugates were synthesized with the use of tert-butyl esters of amino acids or benzyl esters of dipeptides; they contained two residues of L-amino acids (Met, Phe, Pro, and Ile or dipeptides Gly-Leu and Gly-Phe). Activation of GA carboxy groups was carried out with the help of N,N'-dicyclohexylcarbodiimide, N-hydroxysuccinimide, or N-hydroxybenzotriazole with dicyclohexylcarbodiimide. A proline-containing GA derivative is a low-toxic substance; it raises the level of agglutinins by 3.7 times in the blood of mice and 3 times that of hemolysins compared with the control. Dipeptide GA derivatives possess an expressed anti-HIV-1 activity in cultures of MT-4 cells and are 90–70 times less cytotoxic than azidothymidine. The selectivity index of the compounds exceeds those of GA by 110 and 34 times, respectively.

Key words: glycyrrhizic acid, conjugates, amino acids, dipeptides, synthesis, immunostimulating and anti-HIV-1 activity.

DOI: 10.1134/S106816200909040141
To widen the line of nitrogen-containing GA derivatives, we synthesized the derivatives (III)–(XV) with the use of tert-butyl esters of L-amino acids. The application of them considerably simplifies the stage of deblocking of the C-terminal amino acids: it comes down to the treatment of the resulting conjugate with TFA at 20–22°C for 0.5–1 h [8]. The GA carboxy group was activated with the help of a HONSu–DCC mixture in dioxane according to the procedure in [13]. Carrying out this reaction at 0–5°C with the use of 2.5 DCC equiv allows the selective modification of only carboxy groups of GA carbohydrate moiety (II). The interaction of active GA ester (II) with tert-butyl esters of L-amino acids (dipeptides) was carried out at 20–22°C in the presence of a small excess of triethylamine (1–2 mmol) necessary for the temporary protection of the aglycone carboxyl.

Bis(tert-butyl) esters (III) and (IV) with free 30-COOH were purified by CC on silica gel in 50–52% yields. GA identified by TLC was the main impurity in the reaction products. The structures of the compounds were confirmed by spectral methods; in the spectra of 13C NMR of (III) and (IV), there was a signal of the aglycone free COOH group at 180.3–180.7 ppm. Next, the esterification of (III) was carried out by a diazomethane ether solution with the subsequent OBu' deblocking of the formed triester by TFA in CH2Cl2 in the 30-methyl ester (V) in a 65% yield.

The treatment of compounds (III) and (IV) with TFA led to yields of 55–58% of GA derivatives (VI) and (VII) containing two Met or Ile residues, respectively.

Compounds (IX) and (XI) were obtained by activation of GA COOH groups with the help of HONSu–DCC and the use of phenylalanine and proline tert-butyl esters, and the subsequent deblocking of the formed protected conjugates (VIII) and (X) by TFA without preliminary chromatographic purification.

Conjugates (XIII) and (XV) containing dipeptide fragments -Gly-Leu-OH and -Gly-Phe-OH were synthesized by the activation of GA carboxy groups by an HOBt–DCC mixture at 0°C and the subsequent interaction with Gly-Leu and Gly-Phe benzyl esters hydrochlorides at a molar ratio of GA : HOBt : DCC : AA = 1 : 3 : 2.5 : 2.5–3.0 mol/mol. The activation of GA with the help of HONSu–DCC in this case has not led to the desired results, which is probably due to the presence of bulky ester groups in AA.

The obtained benzyl esters of GA conjugates (XII) and (XIV) were reprecipitated from MeOH by ether and, without further purification, deblocked by hydrogenolysis in 75% AcOH in the presence of 10% Pd/C. The target glycodipeptides (XIII) and (XV) were then chromatographed on silica gel columns; yields were 45 and 44%.

The formation of a CONH bond between the NH2 group of AA and COOH groups of the GA diglucuronoside chains was proven by low field signals (172–176 ppm) of the corresponding C=O groups and the presence of signal α-CH atoms of amino acid residues at 51–58 ppm. In the 13C NMR spectrum of GA conjugate with Pro (XI), the signals of the heterocyclic CH group appear at 61.3 and 61.7 ppm.

The acute toxicity of (XI) was investigated on white nonbred mice of 15- to 20-g of body mass at an intraperitoneal injection of the preparation by the Kerber method [14]. This compound did not cause any toxic phenomena at a dose up to 950 mg/kg and can be attributed to the III class of low dangerous substances.

The effect of (XI) on the humoral immune response was investigated on white mice of no breed with body
Table 1. The effect of compound (XI) on the level of agglutinins and hemolysins in sensitized mice

| Compound | After 7 days (10 mg/kg) | After 14 days (2 mg/kg) |
|----------|-------------------------|-------------------------|
|          | Agglutinins (log₂)      | Hemolysins (log₂)       |
| (XI)     | 1.50 ± 0.22*            | 3.40 ± 0.10*            |
| (XVI)    | 1.00 ± 0.20*            | 1.75 ± 0.10*            |
| Control  | 0.40 ± 0.24             | 0.80 ± 0.20             |

Note: *p < 0.05.

The anti-HIV-1 activity of GA dipeptide derivatives (XIII) and (XV) was investigated. The cytotoxic and antiviral activities of preparations were studied in a culture of transferable human T lymphocytes (line MT-4) on a traditional model of acute HIV-1 infection with the use of the HIV-1/EVK strain [16]. GA at a concentration of 100 µg/ml and azidothymidine (Zydovudin, AZT), one of the most known anti-HIV-1 preparations, at concentration of 0.1 µg/ml [17, 18] were used as preparations for comparison. The AZT mechanism of action is connected with the inhibition of the enzyme reverse transcriptase (RT) of HIV, which provides reading of viral DNA in the infected cell from the RNA containing in virion (the transport form of the virus). However, with the passage of time, HIV-1 becomes resistant to the action of RT inhibitors. Therefore, recently AZT and its analogues have begun to be applied together with antiviral preparations with another action mechanism—protease inhibitors. Moreover, unlike GA, AZT is a toxic preparation [19].

The main drawback of GA is its low efficiency as an HIV RT inhibitor [20] and the high doses of the preparation needed to cause inhibition of HIV reproduction [16].

The inhibiting effect of our compounds was estimated on the 4th day of culturing by the EIA method, i.e., by measuring the amount of the virus-specific protein p24, a viral antigen. In addition, the proportion of viable cells was determined by staining with Trypan dark blue dye after counting in a Goryaev chamber. On the basis of the experimental data, dose-dependent curves were plotted and quantitative characteristics of inhibition were determined: ID₉₀, a concentration of a compound that causes a 50% inhibition of the virus production or providing 50% protection of cells against death caused by infection; ID₉₀, a concentration causing 90% inhibition of virus production or providing 90% protection of cells from death due to infection; and IS, a selectivity index, i.e., the ratio of toxic dose CD₅₀ to its effective dose ID₉₀. The results of these experiments are listed in Tables 2–4.

The GA derivative (XII) exhibits anti-HIV-1 activity at the concentration range of 1 µg/ml and ID₉₀ 0.3 µg/ml (Tables 2 and 3). Note that the compound is not toxic for the HIV infected cells within the concentration range studied and did not protect a cell from the cytopathogenic action of the virus. In these experiments, control preparation AZT at a concentration of 0.05 µg/ml provided protection of cells by 97.5% (Table 2). Nevertheless, the IS of (XIII) is 110 times higher than that of GA (Table 3).

Compound (XV) shows expressed anti-HIV-1 activity inhibiting with high efficiency the accumulation of the virus-specific protein p24 (Table 4). Quantitative characteristics of the p24 inhibition by compound (XV) are given in Table 3. This compound possesses 34 times higher IS compared with GA, and ID₉₀ (0.7 µg/ml) on p24 inhibition is 170 times lower in comparison with GA; 90% suppression of the virus reproduction is observed at a (XV) concentration of 0.80 µg/ml. At a concentration of 10 µg/ml, the inhibiting action of this
compound on HIV-1 p24 accumulation was not lower than that of the AZT effect at a concentration of 0.05 µg/ml. Moreover, (XV) appeared to be less toxic than AZT; its CD₅₀ is 250 ± 0.7 µg/ml.

Taking into account that IS values determined for GA vary from 4.45 to 24 [19, 20], it is possible to say that chemical modification of the glycoside by the introduction of dipeptide fragments in the carbohydrate part of molecule led to a substantial increase in anti-HIV-1 activity (an increase in IS and decrease ID₅₀) or a change in cytotoxicity depending on the structure of the dipeptide entered.

Thus, GA conjugation with amino acids or dipeptides is a prospective way of obtaining new anti-HIV-1 agents which exceed the native glycoside in respect to antiviral activity. The GA derivative (XV) was recommended for wide biological studies (Vector State Research Center).

**EXPERIMENTAL**

IR spectra (ν, cm⁻¹) were obtained on a Specord M-80 spectrophotometer in paste with Vaseline oil. UV spectra (λmax, nm (log ε)) were measured on a UF-400 spectrometer in MeOH. ¹H and ¹³C NMR spectra (δ, ppm) were registered on Bruker AM-300 spectrometers with working frequencies of 300 and 75.5 MHz with broadband and out resonance suppression of protons in CD₃OD or DMF-d₆. Tetramethylsilane was used as an internal standard. Optical activity was measured on a Perkin-Elmer 241 MC polarimeter in a tube with an optical length of 1 dm. Melting points were determined on a Boetius microtread.

Thin layer chromatography was carried out on Sorbfil plates (ZAO Sorbpolymer, Russia) using a 45 : 10 : 1 CHCl₃–MeOH–H₂O solvent system. The substances’ spots were detected with a 20% phosphotungstic acid solution in ethanol with the subsequent heating at 110–120°C for 2–3 min. Column chromatography (CC) was carried out on KSK silica gel (fraction 50–150, dry classification) (ZAO Sorbpolymer, Russia). We applied in the work N,N'-dicyclohexylcarbodiimide, N-hydroxysuccinimide, and N-hydroxybenzotriazole (Aldrich), L-amino acids, dipeptides, and tert-butyl esters (hydrochlorides) (Reanal, Hungary). Triethylamine was kept for a day over KOH and distilled. Solvents were evaporated in a vacuum at a temperature of 50–60°C.

**General procedure of obtaining compounds (III), (IV), (VIII), and (X).** HONSu (0.6 g, 5.2 mmol) and DCC (0.55 g, 2.5 mmol) were added to a solution of GA (0.82 g, 1 mmol) in dioxane (10 ml) at 0 to +5°C. The reaction mixture was kept at this temperature for 3 h and left overnight in a refrigerator. The precipitate of N,N'-dicyclohexylurea was filtered off and L-amino acid tert-butyl ester hydrochloride (2.5–3.0 mmol) and Et₃N (0.6–0.7 ml, 4.3–5.1 mmol) were added to the filtrate. The mixture was stirred at 20–22°C for 24 h and diluted with cold water. The precipitate was filtered, washed with water, dried, and chromatographed on a column with silica gel eluted with 100 : 10 : 1 to 50 : 10 : 1 CHCl₃–MeOH–H₂O mixtures. Fractions homogeneous by TLC were combined and evaporated.

**Compound (III); yield 52%; [α]D[20] + 70° (c 0.04; MeOH); IR spectrum: 3600–3200 (OH, NH), 1730 (COOR), 1710 (COOH), 1660 (C=O), 1540 (C=C), 1370 (C–O–C), 1280 (C–O–N), 1170 (C–O–C), 1040 (C–O–C), 750 (C–O–C), 670 (C–O–C), 510 (C–N–C), 460 (C=O), 350 (OH, NH).**

**Table 2. Anti-HIV-1 activity of compound (XIII) on culture of MT-4 cells**

| Preparation          | Concentration of a preparation, µg/ml | Share of viable cells, % | Virus-specific protein p24 accumulation, ng/ml | inhibition, % |
|----------------------|---------------------------------------|---------------------------|-------------------------------------------------|---------------|
| (XIII)               |                                       |                           |                                                 |               |
| 10                   |                                       | 21.43 ± 2.13              | 1829.46 ± 304.77                                | 48.58 ± 8.57  |
| 1                    |                                       | 15.39 ± 4.28              | 1209.30 ± 214.32                                | 66.01 ± 6.04  |
| 0.1                  |                                       | 25.31 ± 3.07              | 2229.98 ± 185.50                                | 37.32 ± 6.04  |
| 0.01                 |                                       | 24.51 ± 4.51              | 2542.09 ± 179.53                                | 28.55 ± 5.05  |
| 0.001                |                                       | 25.39 ± 3.43              | 2710.47 ± 168.38                                | 23.82 ± 4.73  |
| Azidothymidine       | 0.05                                  | 97.50 ± 1.34              | 314.10 ± 61.28                                  | 91.17 ± 4.53  |
| MT-4 (control, uninfected cells) | –                                    | 96.43 ± 1.12              | –                                               | –             |
| MT-4 (HIV-infected cells) | –                                    | 25.93 ± 2.86              | 3557.84 ± 117.34                                | 0             |

**Table 3. Quantitative characteristics of anti-HIV-1 activity of compounds (XIII) and (XV)**

| Compound | CD₅₀ | ID₅₀ |
|----------|------|------|
|          | µg/ml| µM   |
| (XIII)   | 330  | 276.2| 0.3  | 0.25 | 48.58 | 48.58 ± 8.57 |
| (XV)     | 250  | 0.7  | 0.25 | 342.5|
| GA       | 1950 | 125  | 10.3 |
The precipitate was filtered, dried, and kept for 1 h in

\[ \text{MT-4 (control, uninfected cells)} - 96.43 \]

\[ \text{Azidothymidine 0.05 97.50} \]

\[ \text{MT-4 (HIV-infected cells)} - 25.93 \pm 2.86 \]

\[ 3998.54 \pm 101.32 \]

\[ \text{Compound (VI). A solution compound (III) tert-butyl ether (0.5 mmol) in 5 ml of a TFA-CHCl}_2 \text{ mixture (1 : 1) was kept for 1 h at 20–22°C and evaporated.} \]

\[ \text{TLN-biocarcinogenic fractions were combined; yield 55%; [α]_D^{20} + 55° (c 0.02, MeOH); IR spectrum: 3600–3200 (OH, NH), 1710 (COOH), 1650 (C=O), 1550 (CONH); UV spectrum: 249 (4.0); 13C NMR (CD}_2\text{OD): 40.3 (C1), 26.0 (C2), 90.3 (C3), 40.7 (C4), 56.4 (C5), 18.5 (C6), 33.8 (C7), 62.8 (C9), 38.0 (C10), 202.3 (C11), 128.9 (C12), 171.3 (C13), 44.5 (C14), 27.6 (C15), 27.4 (C16), 32.9 (C17), 49.9 (C18), 42.4 (C19), 44.8 (C20), 32.0 (C21), 38.9 (C22), 28.5 (C23), 17.3 (C24), 17.0 (C25), 19.4 (C26), 23.9 (C27), 28.8 (C28), 29.3 (C29), 180.3 (C30), 104.8 (C1’), 183.3 (C2’), 75.3 (C3’), 75.9 (C3”), 73.5 (C4), 77.2 (C5), 77.7 (C5”), 171.7 (C6’), 171.5 (C6”), (Ile): 172.8, 172.7 (COOR), 58.3, 58.0 (α-CH); 34.7, 37.4, 12.2, 12.0 (CH, CH), Found, %: N 2.3, C_{62}H_{100}N_{20}O_{18}S_{2}. Calculated, %: N 2.44. \]

\[ \text{Compound (V). Compound (III) (0.5 g) in 20 ml MeOH was treated with an ethereal solution of diazomethane before a stable yellow color and evaporated. The residue was precipitated from MeOH by ether. The precipitate was filtered, dried, and kept for 1 h in 5 ml of TFA in 5 ml of CHCl}_3. The residue after evaporation was chromatographed on a silica gel column, eluting with CHCl}_3-MeOH–H}_2O mixtures 100 : 10 : 1, 50 : 10 : 1, and 25 : 10 : 1; yield 65%; R_f 0.48; [α]_D^{20} + 35 ± 2° (c 0.04, MeOH); 13C NMR (DMF-d}_6): 38.1 (C1), 25.0 (C2), 89.8 (C3), 55.2 (C5), 17.6 (C6), 33.9 (C7), 45.6 (C8), 61.9 (C9), 36.2 (C10), 200.0 (C11), 128.0 (C12), 170.6 (C13), 43.5 (C14), 32.8 (C17), 48.9 (C18), 41.3 (C19), 44.3 (C20), 32.1 (C21), 37.1 (C22), 27.7 (C23), 16.5 (C25), 18.7 (C26), 23.3 (C27), 27.9 (C28), 28.5 (C29), 177.3 (C30), 51.7 (C31), 104.2 (C1’, C1”), 73.8 (C2”, 72.5 (C4’, C4”), 76.7, 76.5, 75.5 (C5’, C5”), 170.0, 169.8 (C6’, C6”), (Met): 174.2, 174.0 (2 COOH), 52.4, 51.9 (α-CH); 26.7, 26.4, 15.0 (CH, CH, CH) Found, %: N 2.7, S 5.80 C_{53}H_{82}N_{20}O_{18}S_{2}. Calculated, %: N 2.62, S 6.00. \]

\[ \text{Compound (VII) was obtained like (VI) from (IV) tert-butyl ester (0.5 mmol) in a 58% yield; R_f 0.25, [α]_D^{20} + 60 ± 2° (c 0.04; MeOH); IR spectrum: 3600–3200 (OH, NH), 1710 (COOH), 1660 (C11=O), 1540 (CONH); UV spectrum: 248 (3.95); 1H NMR (CD}_2\text{OD): 0.58, 0.65, 0.80, 0.84, 0.96, 1.00, 1.25 (21 H, all s, 7 CH, 4.00–4.19, 15.0–19.0 (CH, CH, CH); 2.25 (1 H, with, H2), 2.70, 2.78, 2.98, 3.00 (s, CH, CH, CH, CH) 2.82 (1 H, c, CH, H18), 3.29 (1 H, d, J 8.9 Hz, H3), 3.57 (1 H, m, CH), 4.42 (1 H, d, J 7.3 Hz, H1”), 4.55 (1 H, d, J 7.8 Hz, H1”).} \]
5.56 (1 H, s, H12), 7.76 (2 H, s, NH). 13C NMR (CD$_2$OD): 40.3 (C1), 27.6 (C2), 90.3 (C3), 40.7 (C4), 56.4 (C5), 18.5 (C6), 33.8 (C7), 46.8 (C8), 63.2 (C9), 38.1 (C10), 202.7 (C11), 126.9 (C12), 171.3 (C13), 44.6 (C14), 27.4 (C15, C16), 33.0 (C17), 42.5 (C19), 44.9 (C20), 32.0 (C21), 38.7 (C22), 28.4 (C23), 17.0 (C24), 17.3 (C25), 19.4 (C26), 23.9 (C27), 28.8 (C28), 29.2 (C29), 180.5 (C30), 104.9 (C1), 105.0 (C1'), 81.7 (C2'), 75.2 (C2''), 76.3 (C3'), 76.0 (C3''), 73.4 (C4'), 73.6 (C4''), 77.3 (C5'), 77.7 (C5''), 172.9, 171.4 (C6', C6''), (ile): 174.2, 173.2 (COOH), 57.9, 57.6 (α-CH), 38.9, 38.7 (CH), 26.5, 26.2 (CH$_2$). Found, %: N 2.5. C$_{54}$H$_{84}$N$_2$O$_{18}$. Calculated, %: N 2.7.

**Compound (IX).** The protected compound (VIII) (0.9 g) without preliminary purification was treated with TFA and then chromatographed on a silica gel column as described above for compound (VI). Yield of (IX) 55%: [α]$_D^{20}$ + 60 ± 2° (c 0.02, MeOH); IR spectrum: 3600–3200 (OH, NH), 1720 (COOH), 1720 (COOH), 1660 (C=O), 1540 (CONH), and 1510 (Ph). Found, %: N 8.0. C$_{15}$H$_{24}$N$_2$O$_3$ - HCl. Calculated, %: N 8.87.

**Glycyl-L-leucine hydrochloride benzyl ester;** yield 60%; [α]$_D^{20}$ + 10° (c 0.04, MeOH); IR spectrum: 1740 (COOR), 1650, 1590 (NH$_3$); 1550 (CONH), 1525 (Ph). Found, %: N 8.51. C$_{15}$H$_{23}$N$_2$O$_3$ - HCl. Calculated, %: N 8.87.

**Glycyl-L-phenylalanine hydrochloride benzyl ester;** yield 66%; IR spectrum: 1730 (COOR), 1640, 1590 (NH$_3$); 1540 (CONH), 1520 (Ph). Found, %: N 7.92. C$_{15}$H$_{23}$N$_2$O$_3$ - HCl. Calculated, %: N 8.03.

**General procedure of obtaining GA derivatives (XI), (XV).**

1. HOBt (0.40 g, 3 mmol) and DCC (0.55 g, 2.5 mmol) were added to a solution of GA (0.82, 1 mmol) in dioxane (20 ml) at 0–5°C. The mixture was stirred at this temperature for 1 h, at 20–22°C for 3 h, and left overnight in a refrigerator. The dicyclohexylurea precipitate was filtered off and Gly-L-LeuOBzl or Gly-L-PheOBzl hydrochloride (2.5–3.0 mmol) and Et$_3$N (0.7 ml) were added to the filtrate during cooling on an ice bath. The reaction mixture was kept for 24 h with periodic stirring at 20–22°C and diluted with cold water; the precipitate was filtered, washed with water, and dried. The products were reprecipitated with ether from methanol; the crude protected compounds (XII) or (XIV) containing dipeptide benzyl esters were obtained and characterized by IR spectra exhibiting intensive maxima in the field of 3600–3200 (OH, NH), 1740 (COOBz1), 1660 (C=O), 1540 (CONH), and 1510 (Ph).

2. The resulting benzyl esters of compounds (XII) and (XIV) (0.5–0.7 mmol in 20 ml of 75% AcOH) were hydrogenated in the presence of 10% Pd/C for 48 h. The catalyst was filtered and solvent was evaporated. The residue was chromatographed on silica gel. Elution with 100 : 10 : 1, 50 : 10 : 1, and 30 : 10 : 1 CHCl$_3$–MeOH–H$_2$O mixtures gave TLC homogeneous fractions which were combined and evaporated.

**Compound (XIII);** Yield 45%; R$_f$ 0.5, [α]$_D^{20}$ + 57 ± 2° (c 0.04; MeOH); IR spectrum: 3600–3200 (OH, NH), 1710 (COOH), 1660 (C=O); UV spectrum: 248 (4.2); 13C NMR (CD$_2$OD): 40.3 (C1), 28.3 (C2), 90.9 (C3), 40.8 (C4), 56.4 (C5), 18.4 (C6), 33.8 (C7), 46.8 (C8), 63.2 (C9), 38.1 (C10), 202.5 (C11), 129.5 (C12), 171.3 (C13), 44.6 (C14), 27.4 (C15), 27.6 (C16), 33.0 (C17), 32.0 (C18), 44.9 (C20), 39.0 (C22), 28.5 (C23), 16.7 (C24), 17.0 (C25), 19.4 (C26), 23.9 (C27), 28.8 (C28), 29.2 (C29), 180.5 (C30), 104.8 (C1), 105.5 (C1'), 81.0 (C2), 75.0 (C2''), 75.3 (C3'), 73.5 (C3''), 73.4 (C4'), 79.4 (C4''), 77.2 (C5'), 77.7 (C5''), 171.5, 170.6 (C6', C6''), (Phe): 173.5, 172.6 (COOH), 54.7, 54.5 (α-CH), 137.7, 130.5, 130.3, 130.0, 129.0, 128.0, 127.8 (Ph). Found, %: N 2.6. C$_{60}$H$_{84}$N$_2$O$_{18}$. Calculated, %: N 2.5.

**General procedure of obtaining glycyll-L-leucine and glycyll-L-phenylalanine benzyl ester hydrochlorides.** A suspension of dipeptide (5 g) in 100 ml of dry benzyl alcohol was treated with freshly distilled SOCl$_2$ (15 ml) and heated at 100°C for 3 h without access to moisture. After cooling the mixture down to 20–22°C, dry ether (200 ml) was added and the mixture was left in a refrigerator before the formation of a precipitate, which was filtered, dried, and recrystallized from methanol.

**Glycyl-L-leucine hydrochloride benzyl ester;** yield 60%; [α]$_D^{20}$ + 10° (c 0.04, MeOH); IR spectrum: 1740 (COOR), 1650, 1590 (NH$_3$); 1550 (CONH), 1525 (Ph). Found, %: N 8.51. C$_{15}$H$_{24}$N$_2$O$_3$ - HCl. Calculated, %: N 8.87.

**Glycyl-L-phenylalanine hydrochloride benzyl ester;** yield 66%; IR spectrum: 1730 (COOR), 1640, 1590 (NH$_3$); 1540 (CONH), 1520 (Ph). Found, %: N 7.92. C$_{15}$H$_{23}$N$_2$O$_3$ - HCl. Calculated, %: N 8.03.
Compound (XV). Yield 44%. R$_f$ 0.45 (A). [α]$_D^{20}$ + 54 ± 2$^\circ$ (c 0.01; MeOH). IR spectrum: 3600–3200 (OH), 1720 (COOH), 1660 (C=O), 1540 (CONH), 1500 (Ph). A UV-spectrum: 245 (4.0). Found, %: N 4.8. C$_6$H$_{10}$O$_2$N$_4$. Calculated, %: C: 4.6. A spectrum of a $^{13}$C-NMR(CD$_3$OD): 40.4 (C1), 27.4 (C2), 90.7 (C3), 40.8 (C4), 56.5 (C5), 18.4 (C6), 33.8 (C7), 46.8 (C8), 63.2 (C9), 38.1 (C10), 202.5 (C11), 129.2 (C12), 171.4 (C13), 44.6 (C14), 27.6, 27.5 (C15, C16), 32.9 (C17), 49.9 (C18), 42.5 (C19), 44.9 (C20), 32.0 (C21), 38.7 (C22), 28.2 (C23), 17.1, 16.6 (C25, C24), 19.4 (C26), 23.9 (C27), 29.1 (C28), 29.6 (C29), 179.3 (C30), 105.2 (C1'), 106.1 (C1''), 84.1 (C2'), 73.0 (C2''), 75.8 (C3'), 73.5 (C3''), 72.3 (C4'), 72.4 (C4''), 77.3 (C5'), 77.7 (C5''), 169.8 (C6'), 170.1 (C6''), Gly-Phe: 174.6, 172.6 (COOH), 55.1, 55.0 (α-CH); 37.9, 36.7, 30.0, 36.2 (CH2), 138.1, 130.5, 130.4, 129.5, 127.9 (Ph).

Immunotropic activity of (XI) was studied on white mice of no breed of 18- to 20-g in weight. Experiments were carried out on three groups of animals (14 mice in each group, divided into two subgroups with seven mice in each) during the sensitization of mice with a 3% SE suspension introduced intraperitoneally (0.5 ml to each mouse). From the first day of the sensitization, the animals obtained a compound under study (0.5 ml to each mouse). From the first day of the sensitization, the animals obtained a compound under study (0.5 ml to each mouse). On the 7th and 14th days of the sensitization, the animals were decapitated, their blood was collected, and 10 (7-day introduction) and 2 mg/kg (14-day introduction). Control animals were introduced with a 3% SE suspension introduced intraperitoneally (without virus) were diluted with a growth cultural medium up to the sowing concentration of 5×10$^5$ cells/ml and placed in wells of 96-well plates. Then, solutions of the compound under study were brought into the corresponding wells (three wells per each dilution). Final concentrations in the cellular suspension of the preparations studied were from 0.1 to 100 µg/ml.

The inhibiting effect of a compound was estimated on the fourth day of culturing by measuring the quantity of the viral antigen, virus-specific protein p24, and by EIA. In addition, the proportion of viable cells was determined after staining by Trypan Dark Blue and counting in the Goryaev chamber. On the basis of the experimental data, a curve of dose dependence was plotted and the quantitative characteristics of inhibition were determined: ID$_{50}$, a concentration of a compound that inhibits by 50% the production of virus or provides 50% protection of cells against destruction due to infection; ID$_{90}$, a concentration of a compound that inhibits by 90% the production of virus or provides 90% protection of cells against destruction due to infection; IS, a selectivity index, the ratio of toxic dose CD$_{50}$ to the effective dose ID$_{50}$.

ACKNOWLEDGMENTS

Biological activity and toxic and pharmacological properties of new GA derivatives were investigated on the Faculty of Pharmacology of Bashkortostan State Medical University, in the Laboratory of New Medical Products of Institute of Organic Chemistry of the Ufa Scientific Center of the Russian Academy of Sciences (acute toxicity, immunotropic properties) and Institute of Molecular Biology of the Vector State Research Center of Virology and Biotechnology (anti-HIV-1 activity).

This work was supported by the Russian Foundation for Basic Research (project no. 08-03-13514 ofi-ts).

REFERENCES

1. Tolstikov, G.A., Baltina, L.A., Grankina, V.P., Kondratenko, R.M., and Tolstikova, T.G., Solodka: bioreaznoobrazie, khimiya, primenenie v medicine (Loricore: Biodiversity, Chemistry, and Application in Medicine), Novosibirsk: Akadem. Izd. Geo, 2007.
2. Tolstikov, G.A., Baltina, L.A., Shul’ts, E.E., and Prokovskii, A.G., Bioorg. Khim., 1997, vol. 32, pp. 5–14.
3. De Clerq, E., Med. Res. Rev., 2000, vol. 20, pp. 323–349.
4. Iino, S., Tango, T., Matsushima, T., Toda, G., Miyaka, K., Hino, K., Kumada, H., Yasuda, K., Kuroki, T., Hirayama, C., and Suzuki, H., Hepatol. Res., 2001, vol. 19, pp. 31–40.
5. Okuno, T., Ara, K., and Shindo, M., Ipn. J. Clin. Med., 1995, vol. 53, pp. 1022–1025.
6. Ito, M., Nakashima, M., Baba, M., Pauwels, R., De Clercq, E., Shigeta, Sh., and Yamamoto, N., Antiviral Res., 1987, vol. 7, pp. 127–137.
7. Baltina, L.A., Sakhuntadinova, G.M., Zarudii, F.S., Lazareva, D.N., Tolstikov, G.A., and Davydova, V.A., Khim.-Farm. Zh., 1990, vol. 24, pp. 119–121.
8. Baltina, L.A., Ryzhova, S.A., Vasil’eva, E.V., Tolstikov, G.A., Sakhuntadinova, G.M., and Zarudii, F.S., Bioorg. Khim., 1994, vol. 20, pp. 1365–1373.
9. Kondratenko, R.M., Baltina, L.A., Vasil’eva, E.V., Baschenko, N.Zh., Friedman, S.M., Baltina, L.A., Jr., and Tolstikov, G.A., Bioorg. Chem., 2006, vol. 30, pp. 168–173 [Russ. J. Bioorg. Chem. (Engl. Transl.), vol. 30, pp. 148–153].
10. Kondratenko, R.M., Baltina, L.A., Jr., Baltina, L.A., Baschenko, L.N., and Tolstikov, G.A., Bioorg. Chem., 2006, vol. 32, pp. 660–666 [Russ. J. Bioorg. Chem. (Engl. Transl.), vol. 32, pp. 595–601].
11. Kondratenko, R.M., Baltina, L.A., Plyasunova, O.A., Pokrovskii, A.G., and Tolstikov, G.A., RF Patent No. 2198177, Byul. Izobret., 2003, no. 4.
12. Tolstikov, G.A., Baltina, L.A., Pokrovskii, A.G., Plyasunova, O.A., and Sandakhchiev, L.S., RF Patent No. 2024546, Byul. Izobret., 1994, no. 23.
13. Kondratenko, R.M., Baltina, L.A., Vasil’eva, E.V., Baltina, L.A., Jr., Ismagilova, A.F., Nasyrov, Kh.H., Baschenko, N.Zh., Kireeva, R.M., Friedman, S.I., and Tolstikov, G.A., Bioorg. Chem., 2004, vol. 30, pp. 61–67 [Russ. J. Bioorg. Chem. (Engl. Transl.), vol. 30, pp. 53–59].
14. Elizarova, O.N., Opredelenie porogovykh doz promyshlennyykh yadov pri peroral’nom vvedenii (Determination of Threshold Doses of Perorally Administered Industrial Toxicants), Moscow: Meditsina, 1971, pp. 47–48.
15. Kondratenko, R.M., Baltina, L.A., Vasil’eva, E.V., Nasyrov, Kh.M., Kireeva, R.M., Baschenko, N.Zh., Friedman, S.M., Baltina, L.A., Jr., and Tolstikov, G.A., Bioorg. Chem., 2004, vol. 30, pp. 168–173 [Russ. J. Bioorg. Chem. (Engl. Transl.), vol. 30, pp. 148–153].
16. Plyasunova, O.A., Egoricheva, I.N., Feduk, N.V., Pokrovskii, A.G., Baltina, L.A., Murinov, Yu.I., and Tolstikov, G.A., Vopr. Virusol., 1992, nos. 5–6, pp. 235–238.
17. Mashkovskii, M.D., Lekarstvennye sredstva v 2-kh t., Izd. 14-e, pererab., ispr. dop. (Drugs), 14th ed. (rev. suppl. correct.), Moscow: Novaya Volna, 2000.
18. Rossiiskii terapevticheskii spravochnik (Russian Therapeutic Handbook), Moscow: GEOTAR-Media, 2005.
19. Plyasunova, O.A., Mamaeva, O.A., Gashnikova, N.M., Feduk, N.V., and Pokrovskii, A.G., VICH/SPID Rodstv. Probl., 1999, vol. 3, p. 58.
20. Plyasunova, O.A., Screening and Study of Drugs against Human Immunodeficiency Virus, Cand. Sci. (Biol.) Dissertation, Kol’tsovo: Gos. Nauch. Tsentr Vektor, 1992.
21. Kondratenko, R.M., Baltina, L.A., Mustafina, S.R., Makarova, N.V., Nasyrov, Kh.M., and Tolstikov, G.A., Khim.-Farm. Zh., 2001, vol. 35, pp. 39–42.