ISSN 1068-1620, Russian Journal of Bioorganic Chemistry, 2016, Vol. 42, No. 2, pp. 191–197. © Pleiades Publishing, Ltd., 2016.
Original Russian Text © M.V. Kozlov, A.A. Kleymenova, K.A. Konduktorov, A.Z. Malikova, K.A. Kamarova, R.A. Novikov, S.N. Kochetkov, 2016, published in Bioorganicheskaya Khimiya, 2016, Vol. 42, No. 2, pp. 214–220.

Synthesis of (*Z*)-*N*-Hydroxy-3-Methoxy-3-Phenylacrylamide as New Selective Inhibitor of Hepatitis C Virus Replication

M. V. Kozlov[1], A. A. Kleymenova, K. A. Konduktorov, A. Z. Malikova, K. A. Kamarova, R. A. Novikov, and S. N. Kochetkov

Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, ul. Vavilova 32, Moscow, 119991 Russia

Received July 1, 2015; in final form, August 7, 2015

Abstract—According to recently published results, cinnamic hydroxamic acid (CHA) inhibits replication of hepatitis C virus (HCV). We synthesized a structural analogue of CHA, i.e., (*Z*)-*N*-hydroxy-3-methoxy-3-phenylacrylamide, which inhibited HCV replication five times more selectively than CHA. It was found that both compounds did not inhibit deacetylation of Ac-α-tubulin with histone deacetylase 6, the activity of which is important for virus replication.

Keywords: cinnamic hydroxamic acid, hepatitis C virus, histone deacetylase 6, Ac-α-tubulin

DOI: 10.1134/S1068162016010076

INTRODUCTION

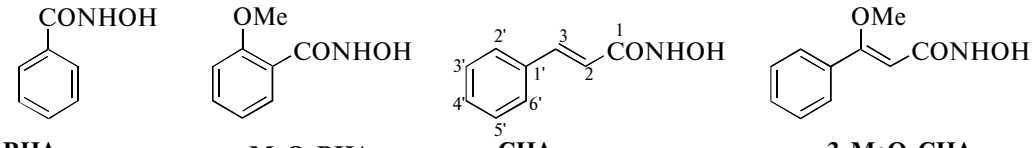
Currently, therapy of hepatitis C is based on direct action drugs, which efficiently inhibit viral proteins and enzymes. A widely used drug Harvoni (Gilead Sciences, Inc., United States) is a mixture of blocker of the NS5A protein and the inhibitor of RNA-dependent RNA and NS5B polymerase of the virus. The sustained antiviral response of this drug is observed in 90% of patients [1–3]. The relatively short duration of the treatment (12 to 24 weeks) reduces the occurrence of cross-resistant forms of the virus in the patient's body. However, taking into account the number of HCV-infected patients (about 200 million people), the number of patients immune to Harvoni may reach 20 million people. Therefore, the search for new therapeutic targets remains relevant, especially as regards proteins of the host cell, which play an important role in intracellular virus reproduction.

According to literature data, hydroxamic acids can selectively inhibit the propagation of HCV replicon in culture of human hepatocytes [4–10]. Among studied compounds, two classes of biologically active hydroxamates can be distinguished, i.e., inhibitors of matrix metalloproteinases [4, 5, 9] and histone deacetylases [6, 8]. Probably, the anti-HCV activity of benzoic, cinnamic, and pyridine hydroxamic acids described in the literature is also associated with inhibition of enzymes of these two classes; however, the mechanism of the inhibition of the HCV replication is still unclear [7, 9, 10].

In 2013, we found that, in contrast to benzohydroxamic acid (BHA, SI =14 and EC_{50} = 12 μM), its *ortho*-methoxy-derivative demonstrated a 4-fold increase in the selectivity while maintaining the activity (*o*-MeO-BHA, SI = 53 and EC_{50} = 12 μM) [7]. Taking into account both these data and the high anti-HCV activity of cinnamic hydrozamic acid (CHA, EC_{50} = 0.86 μM [9]), we decided to synthesize the 3-methoxy analogue of CHA (3-MeO-CHA), which combined the structural elements of *o*-MeO-BHA and CHA and to study the antiviral characteristics of this compound (see formulas).

Abbreviations: BHA, benzohydroxamic acid; CHA, cinnamic hydroxamic acid; HDAC6, histone deacetylase 6; HCV, hepatitis C virus.

[1] Corresponding author: phone: +7 (499) 135-0590; fax: +7 (499) 135-1405; e-mail: kozlovmv@hotmail.com.



BHA
EC_{50} = 15 μM
SI = 14

***o*-MeO-BHA**
EC_{50} = 12 μM
SI = 53

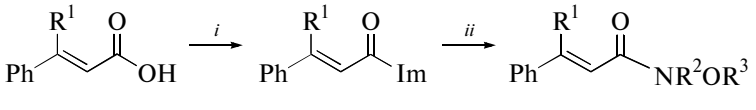
CHA
EC_{50} = 0.86 μM
SI = 45

3-MeO-CHA

Formulas. Molecular structure of 3-MeO-CHA containing structural elements of *o*-MeO-BHA and CHA.

RESULTS AND DISCUSSION

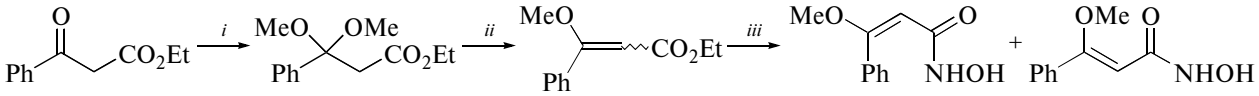
The synthesis of cinnamic hydroxamic acid (CHA) and its monomethyl derivatives (**I**)–(**III**) is carried out by activation of the acid component with carbonyl diimidazole in dimethylformamide, followed by the condensation with hydroxylamine component (Scheme 1). Imidazole is formed during the activation of the initial acid while synthesizing hydroxamic acid and acts then as a base that deprotonates hydrochloride. After evaporation of the solvent, the products were extracted by ethyl acetate from water and were purified by silica gel chromatography. The yields were 20–30%.



Comp.	R^1	R^2	R^3
CHA	H	H	H
(I)	Me	H	H
(II)	H	Me	H
(III)	H	H	Me

Scheme 1. Synthesis of CHA and compounds (**I**)–(**III**). (*i*), CDI/DMF; (*ii*), $NHR_2OR_3 \cdot HCl$; Im, imidazole residue.

To synthesize the 3-methoxy analogue of CHA (**VII**), we used commercially available ethyl benzoylacetate as the starting compound (Scheme 2). The boiling of this β-ketoester for 50 min in a methanol-trimetyl orthoformate mixture in the presence of catalytic amount of TsOH gave dimethylketal (**IV**), which was evaporated and isolated by extraction with *n*-hexane in a yield of 92%.



(IV) **(V)** **(VI)** 3-MeO-CHA (**VII**)

Scheme 2. Synthesis of (*E*)- and (*Z*)-*N*-hydroxy-3-methoxy-3-phenylacrylamides (**VI**) and (**VII**). (*i*), $HC(OCH_3)_3$/TsOH/MeOH/Δ; (*ii*), Δ; (*iii*), $NH_2OH \cdot HCl$/KOH/MeOH.

Examples of thermal enolization of ketals to corresponding vinyl esters are known from the literature [11]. Similarly, pyrolysis of compound (**IV**) led to the mixture of (*E*/*Z*)-isomers of ethyl-3-methoxy-phenylacrylate (**V**) (Scheme 2). The reaction proceeds at 190°C for 15 min with a quantitative yield. The PMR spectrum in Fig. 1 showed that the proton signals of two isomers were grouped in the regions of the proton chemical shifts of the aromatic ring, exocyclic double C–C bond, the methyl residue of enol ester, and ethyl ester residue. The ratio of the intensity of two signals inside each group is constant (about 1/3.4) and corresponds to the content of isomers in the mixture (23 and 77%).

At the final stage of the synthesis, a cooled methanol solution of NH_2OH (2 equiv.) and KOH (1 equiv.) was added to the reaction mixture containing compound (**V**), and the mixture was incubated at room temperature for 18 h. The solution was neutralized, the solvent was evaporated, and an aqueous base (pH ~8) was added to the residue, followed by washing with hexane. The products were extracted with methylene chloride. Hydroxamic acids (**VI**) and (**VII**) were separated from each other and from an unidentified side product by silica gel chromatography.

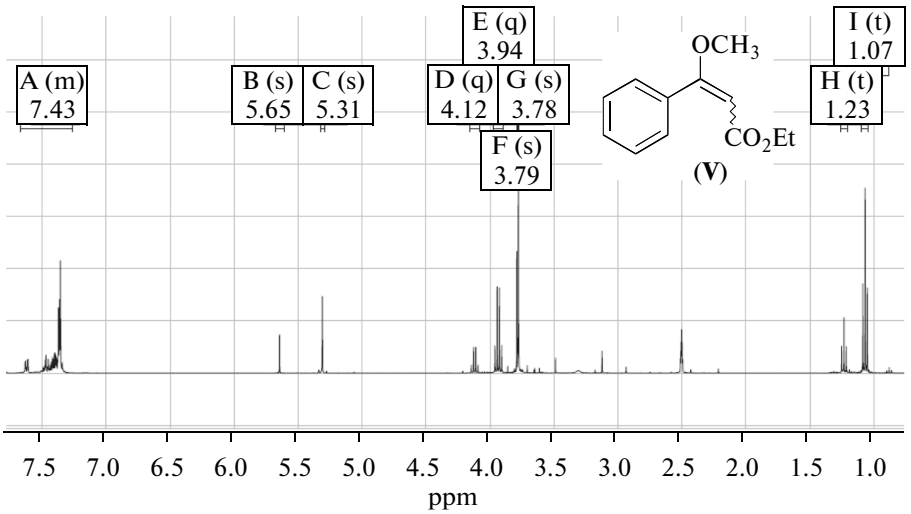


Fig. 1. ^{1}H NMR spectrum of ethyl 3-methoxy-3-phenylacrylate (**V**) (*E*/*Z*)-isomer mixture. Multiplet A, phenyl residue protons; singlets B/C, alkene protons at C2; quadruplets D/E and triplets H/I, protons of the ester ethyl groups; and singlets F/G, protons of enol ether methyl groups.

PMR spectra of compounds (**VI**) and (**VII**) after chromatographic purification are presented in Fig. 2. The signal of C2 proton in compound (**VI**) (5.23 ppm) is shifted upfield by 0.28 ppm compared with that in compound (**VII**) (5.51 ppm). A similar difference in chemical shifts (0.34 ppm) is observed between the main (5.31) and minor (5.65 ppm) signals of C2 protons in the PMR spectrum of isomers (**V**) (Fig. 1). Thus, hydroxamate (**VI**) is the product of the conversion of the ester, the content of which is 77% in the mixture. This means that the yields of compounds (**VI**) and (**VII**) are 10 and 18%, respectively.

The absolute configuration of each product was determined by 2D ^{1}H-^{1}H NOESY NMR spectroscopy. The spectrum of compound (**VI**) contains cross-pecks at 5.23–3.69 ppm (data not shown), which indicates that the C2 protons and protons of 3-MeO group are located on one side of the double bond. This is possible only for the (*E*)-isomer (Fig. 2). On the other hand, the spectrum of compound (**VII**) contains cross-peaks at 5.51–7.54 ppm (data not shown), which indicates the interaction of the C2 proton with protons of the aromatic ring (H2' and H6'), which can occur only in the (*Z*)-isomer (Fig. 2).

At the next stage, we examined all prepared compounds as inhibitors of the HCV replication. CHA and compound (**I**) demonstrate close values of antiviral activity (EC_{50} = 2.0 and 2.3 μM, respectively) and cytotoxicity (CC_{50} = 20 μM for both compounds) (table). These values are in satisfactory agreement with published data (EC_{50} = 0.86 and 3.2 μM, respectively) despite the authors use of hepatocytes of the Huh-7/HCV1b-Rluc line [9], which maintain the reproduction of the subgenomic HCV replicon. However, the cytotoxicity values significantly differ from the published data (CC_{50} = 39 and 59 μM, respectively). A possible reason for this discrepancy is the difference in culture media and methods of the cytotoxicity evaluation which were used in our work and in [9].

The data in the table show that methylation of the hydroxamic residue dramatically reduces the antiviral activity and selectivity of *N*-methylated compound (**II**) (EC_{50} = 75 μM and SI = 3.3) and makes *O*-methylated compound (III) completely inactive (EC_{50} > 300 μM). These results imply that the binding strength between the inhibitor and the target protein is determined by the chelating ability of the hydroxamic residue.

Most likely, the spatial proximity of the phenyl ring to the hydroxamic acid residue in (*E*)-isomer (**VI**) sterically hinders the chelation of the metal ion in the active center of the target protein. This is confirmed by a 30-fold decrease in the antiviral activity of this compound in comparison with the activity of (*Z*)-isomer (**VII**) (EC_{50} = 180 and 5.7 μM, respectively). At last, the methoxy group at position 3 of compound (**VII**) increases the selectivity of its action by a factor of almost five compared to CHA, but unfortunately, this group decreases simultaneously the antiviral efficiency by a factor of three (EC_{50} = 5.7 and 2.0 μM, respectively). This result shows that the structural analogies between benzoic and cinnamic hydroxamic acids as HCV inhibitors are unjustified and cannot be used in the future.

Histone deacetylase 6 (HDAC6) is known to be localized in mammalian cells mostly in cytoplasm, where it binds to the tubulin cytoskeleton and deacetylates the acetylated Ac-Lys40 residue of α-tubulin (Ac-α-tubulin) in microtubes [13]. The evaluation of the level of Ac-α-tubulin by western-blotting makes it possible to monitor the cytoplasmic activity of HDAC6. In recent papers, we conformed the direct correlation between the inhibition of the HDAC6 activity and the inhibition of the HCV virus replication by *para*-derivatives of benzhydroxamic acid [8, 10]. CHA is known to selectively inhibit the HDAC6

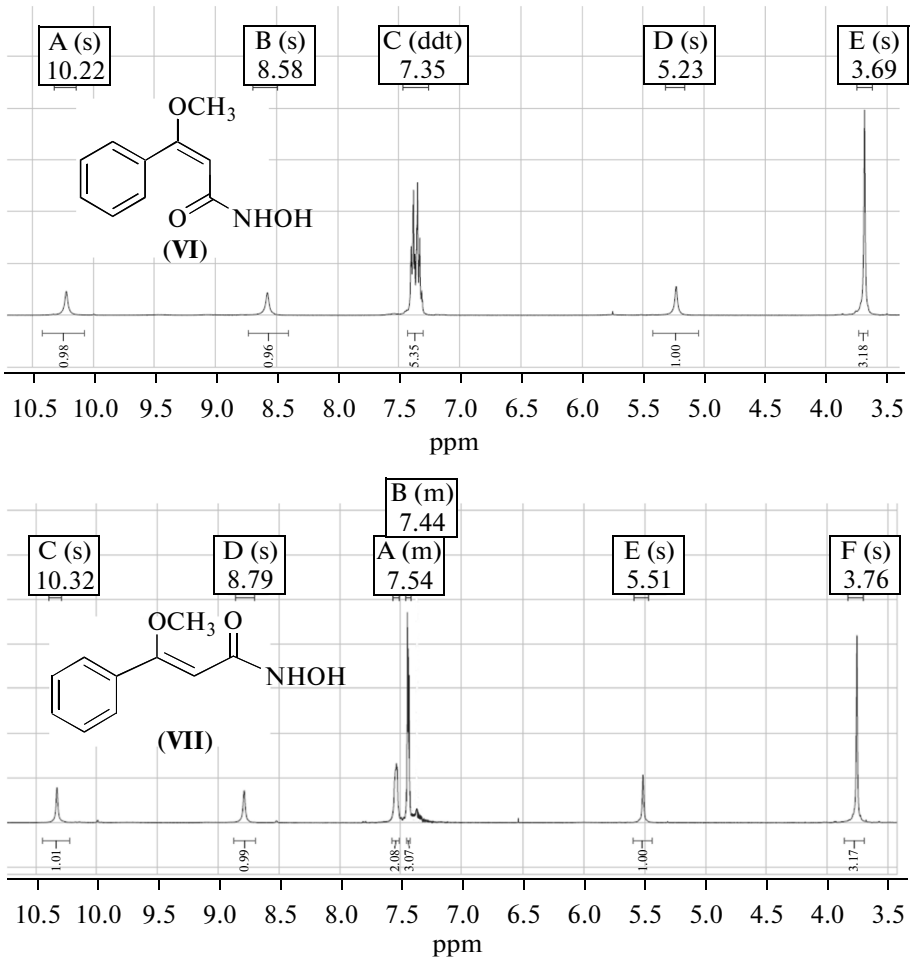


Fig. 2. ^{1}H NMR spectra of (*E*)- and (*Z*)-*N*-hydroxy-3-methoxy-3-phenylacrylamide (**VI**) and (**VII**).

activity (IC_{50} = 22 nM) [12]. However, the incubation of the Huh7-luc/neo cell line in the presence of CHA or compound (**VII**) at concentrations up to 10 or 100 μM, respectively, is not accompanied by any noticeable accumulation of Ac-α-tubulin (data not shown).

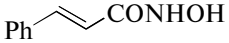
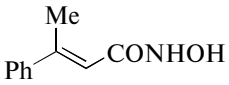
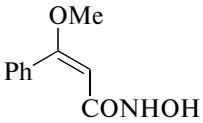
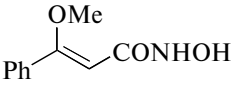
It is hard to imagine that when penetrating into cells, CHA can no longer bind to the active center of HDAC6 at concentrations, which is 500 times higher than the IC_{50} value. The result can most likely be explained by a phenomenon of *substrate selectivity* of this inhibitor. The fact is that the real biological substrate of HDAC6 is Ac-α-tubulin, i.e., the protein with a molecular weight of about 50 kDa but not the peptide used for testing in vitro [12]. The formation of the Ac-α-tubulin–HDAC6 complex can be crucial for the ability of CHA to compete for the catalytic center with ε-*N*-acetylated Lys40 residue. At the same time, CHA may retain its ability to inhibit deacetylation of other protein substrates of HDAC6 and, thereby, to block the HCV replication [8, 10]. If the assumed *substrate selectivity* of CHA as the HDAC6 inhibitor will be confirmed, this will expand the list of examples of *substrate-selective* inhibition, which is known now for kinase p38 MAPK [14], cyclooxygenase COX-2 [15], and kinase PDK-1 [16].

As a result of this work, we have obtained new data showing that the anti-HCV activity of CHA is due to metal ion chelation in the active center of the target protein and this chelation is possible only when the phenyl and hydroxamic groups are located in *trans*-position relative to the double C–C bond of the exocyclic residue. We have synthesized a new HCV inhibitor, (*Z*)-*N*-hydroxy-3-methoxy-3-phenylacrylamide, and have found that the 3-methoxy substituent increases the selectivity of the antiviral effect of the obtained compound by a factor of five as compared to CHA. The fact that Ac-α-tubulin is not accumulated during incubation of cells with Huh7-luc/neo in the presence of CHA gives reason to believe that this hydroxamate possesses *substrate selectivity* relative to HDAC6.

EXPERIMENTAL

We used 1,1'-carbonyl diimidazole (CDI), hydroxylamine hydrochloride, *N*-methyl hydroxylamine

Results of anti-HCV test of structural analogues of CHA

Compound	Structure	EC_{50}, μM	CC_{50}, μM	SI
CHA		**2.0**/0.86*	**20**/39*	**10**/45*
(I)		**2.3**/3.2*	**20**/59*	**8.7**/18*
(II)		**75**	**250**	**3.3**
(III)		**>300**	**>1000**	–
(VI)		**180**	**700**	**3.9**
3-MeO-CHA (**VII**)		**5.7**	**280**	**49**

*Data published in [9].

hydrochloride, methoxylamine hydrochloride, benzoyl acid ethyl ester, trimethyl orthoformate, toluene sulfonic acid monohydrate (TsOH · H_2O), triethylamine (NEt_3), *N*-diisopropylethylamine (DIEA), and dimethylformamide (DMF) (Sigma-Aldrich, United States). TLC was performed on Kieselgel 60 F254 plates (Merck, Germany) in systems $CHCl_3$–EtOH, 10 : 1 (A); $CHCl_3$–EtOH, 20 : 1 (B); $PhCH_3$–MeCN, 20 : 1 (C).

NMR spectra (δ, ppm; *J*, Hz) were recorded on a AMX III-400 spectrometer (Bruker, Germany) with 400 MHz for ^{1}H NMR (internal standard, Me_4Si; solvent, DMSO-d_6) and 100.6 MHz for ^{13}C NMR (solvent DMSO-d_6, suppression of carbon–proton interaction).

CHA and compounds (I)–(III) were synthesized by the same method. The starting acid (4 mmol) was dissolved in DMF (4 mL), followed by the addition of 1,1'-carbonyldiimidazole (0.65 g, 4 mmol). After incubation for 2 h, the corresponding hydroxylamine hydrochloride derivative (8 mmol) was added, and the reaction mixture was stirred until its complete dissolution. After incubation for 18 h, the solvent was evaporated, water (15 mL) was added to the residue, followed by washing with EtOAc (2 × 15 mL). Organic fractions were combined, dried over Na_2SO_4, and evaporated. The products were isolated in yields of 20–30% by silica gel chromatography using a chloroform-ethanol mixture as eluent.

Cinnamic acid *N*-hydroxylamide (CHA), R_f 0.29 (A). ^{1}H NMR spectrum: 10.73 (1H, s, NH), 9.00 (1H, s, OH), 7.55 (2H, d, *J* 6.9 Hz, H2' and H6'), 7.46 (1H, d, *J* 15.9 Hz, H3), 7.43–7.34 (4H, m, H3, H3', H4' and H5'), 6.47 (1H, d, *J* 15.8 Hz, H2). ^{13}C NMR spectrum: 162.68 (C1), 138.28 (C3), 134.78 (C1'), 129.38 (C'4), 128.87 (C'3 and C'5), 127.41 (C'2 and C'6), 119.08 (C2).

(*E*)-*N*-hydroxy-3-phenyl-2-butenamide (I), R_f 0.37 (A). ^{1}H NMR spectrum: 11.25–8.29 (2H, m, N<u>H</u>O<u>H</u>), 7.48 (2H, d, *J* 7.4, H2' and H6'), 7.44–7.33 (3H, m, H3', H4' and H5'), 6.04 (1H, s, H2), 2.49 (3H, s, C<u>H</u>$_3$). ^{13}C NMR: 163.89 (C1), 148.08 (C3), 142.03 (C1'), 128.48 (C'3 and C'5), 128.43 (C'4), 125.78 (C'2 and C'6), 117.22 (C2), 16.66 (<u>C</u>H$_3$).

Cinnamic acid *N*-hydroxy-*N*-methylamide (II), R_f 0.38 (B). ^{1}H NMR spectrum: 10.06 (1H, s, OH), 7.64 (2H, d, *J* 6.8, H2' and H6'), 7.50 (1H, d, *J* 15.9, H3), 7.46–7.34 (3H, m, H3', H4' and H5'), 7.23 (1H, d, *J* 15.9 Hz, H2), 3.22 (3H, s, NC<u>H</u>$_3$). ^{13}C NMR: 165.56 (C1), 140.74 (C3), 134.93 (C1'), 129.57 (C'4), 128.84 (C'3 and C'5), 127.76 (C'2 and C'6), 117.28 (C2), 36.04 (N<u>C</u>H$_3$).

Cinnamic acid *N*-hydroxy-*N*-methylamide (III), R_f 0.38 (B). ^{1}H NMR spectrum: 11.27 (1H, s, NH), 7.64–7.46 (3H, m, H2', H6' and H3), 7.46–7.35 (3H, m, H3', H4' and H5'), 6.42 (1H, d, *J* 15.9, H2), 3.67 (3H, s, OC<u>H</u>$_3$). ^{13}C NMR: 165.62 (C1), 139.51 (C3), 134.56 (C1'), 129.65 (C'4), 128.87 (C'3 and C'5), 127.56 (C'2 and C'6), 118.51 (C2), 63.31 (O<u>C</u>H$_3$).

Ethyl-3,3-dimethoxy-3-phenylpropionate (IV). Trimethyl orthoformate (20 mL) and TsOH · H_2O (500 mg) were added to the solution of ethyl benzoylacetate (5.76 g, 30 mmol) in methanol (25 mL), and the mixture was boiled for 50 min. The reaction mixture was cooled to room temperature, followed by the addition of NEt_3 (0.27 mL). After evaporation of the solvent, the residue was washed with *n*-hexane (50 and 25 mL). The combined organic extracts were successively washed with 2% $NaHCO_3$ (50 mL) and water (50 mL) and dried over Na_2SO_4. The solvent was evaporated, and dimethylketal (IV) was obtained in a yield of 6.57 g (92%) and was used without additional purification. R_f 0.58 (C). ^{1}H NMR: 7.42–7.27 (5H, m, H2'-H6'), 3.78 (2H, q, *J* 7.1, OC$\underline{H}_2$CH$_3$), 3.13 (6H, s, OC$\underline{H}_3$), 2.94 (2H, s, C$\underline{H}_2$CO$_2$Et), 0.88 (3H, t, *J* 7.1, OCH$_2$C$\underline{H}_3$). ^{13}C NMR: 167.68 (C1), 139.54 (C1'), 127.74 (C4'), 127.65 (C'3 and C'5), 126.62 (C'2 and C'6), 100.98 (C3), 59.36 (O$\underline{C}$H$_2$CH$_3$), 48.36 (O$\underline{C}$H$_3$), 42.81 (C2), 13.57 (OCH$_2\underline{C}$H$_3$).

(*E*/*Z*)-Ethyl-3-methoxy-3-phenylacrylate (V) was synthesized by boiling ethyl-3,3-dimethoxy-3-phenylpropionate (IV) (6.11 g, 25.7 mmol) at 190°C for 15 min. A mixture of *E*/*Z* isomers (V) (5.29 g, 100%) was obtained in a 3.4 : 1 ratio and was used for subsequent reaction. ***E*-isomer**: R_f 0.43 (C). ^{1}H NMR spectrum (fragment): 5.31 (1H, s, H2), 3.94 (2H, q, *J* 7. 1, C$\underline{H}_2$CH$_3$), 3.78 (3H, s, OCH$_3$), 1.07 (3H, t, *J* 7.1, CH$_2$C$\underline{H}_3$). ***Z*-isomer**: R_f 0.43 (C). ^{1}H NMR spectrum (fragment): 5.65 (1H, s, H2), 4.12 (2H, q, *J* 7.1, C$\underline{H}_2$CH$_3$), 3.79 (3H, s, OCH$_3$), 1.23 (3H, t, *J* 7.1, CH$_2$C$\underline{H}_3$).

(*E*/*Z*)-*N*-Hydroxy-3-methoxy-3-phenylacrylamide (VI and VII). NH_2OH · HCl (2.82 g, 39.6 mmol) in MeOH (15 mL) and KOH (3.36 g, 60 mmol) in MeOH (9 mL) were dissolved under heating to 50°C. The second solution was added to the first one under stirring, and the mixture was immediately cooled to 0°C. After 10 min, the precipitated KCl was separated by filtration, and the mixture of *E*/*Z*-izomers (**V**) was added to the resultant solution. After incubation for 6 h, another portion of KOH (1.10 g, 19.6 mmol) was added to the reaction mixture, and the reaction was left to proceed overnight, followed by the addition of AcOH (2 mL) and evaporation. The residue was dissolved in H_2O (35 mL) and after the addition of 5 M KOH (~1 mL to pH 8), it was washed with *n*-hexane (30 mL) and CH_2Cl_2 (2 × 35 mL). Fractions containing methylene chloride were combined, dried over Na_2SO_4 and evaporated. Isomers of hydroxamic acids were separated by silica gel chromatography using a CH_2Cl_2–MeOH mixture (20 : 1). ***E*-isomer (VI)** was obtained in a yield of 0.60 g (10%). R_f 0.14 (B), ^{1}H NMR spectrum: 10.22 (1H, s, OH), 8.58 (1H, s, NH), 7.47–7.24 (5H, m, H2'-H6' Phe), 5.23 (1H, s, 2H), 3.69 (3H, s, OCH$_3$). ^{13}C NMR: 163.68 (C1 and C3), 135.34 (C1'), 128.84 (C'4), 128.58 (C'3 and C'5), 127.24 (C'2 and C'6), 92.86 (C2), 55.68 (O$\underline{C}$H$_3$). ***Z*-isomer (VII)** was obtained in a yield of 0.31 g (18%). R_f 0.23 (B), ^{1}H NMR spectrum: 10.32 (1H, s, OH), 8.79 (1H, s, NH), 7.55–7.53 (2H, m, H2' and H6' Phe), 7.46–7.41 (3H, m, H3', H4' and H5' Phe), 5.51 (1H, s, H2), 3.76 (3H, s, OCH$_3$). ^{13}C NMR: 162.52 (C1 or C3), 161.82 (C1 or C3), 134.88 (C1'), 129.69 (C'4), 128.55 (C'3 and C'5), 126.58 (C'2 and C'6), 101.14 (C2), 59.71 (O$\underline{C}$H$_3$).

Cell cultures. Cells of the Huh7 line were grown in a DMEM + F12 (2 : 1) medium containing 10% fetal bovine serum (Hy Clone), 2 mM *L*-glutamine, penicillin (100 U/mL), and streptomycin (0.1 mg/mL) at 37°C and 5% CO_2. Cells were passaged by 1 : 3 or 1 : 5 diluting every three days. Cells of the Huh7-luc/neo lines were grown under the same conditions in the presence of additional antibiotic geneticin (G418, 0.33 mg/mL).

Antiviral activity. The Huh7-luc/neo cells were passaged into 48-well plate (without G418). After incubation for 24 h (40–50% confluence), the studied compounds at different concentrations were added to the cell culture. After three days of incubation at 37°C and 5% CO_2 (monolayer in the control probe was 100%), the medium was removed, cells were washed with PBS and lysed. Luciferase activity of the reference protein was evaluated using the Luciferase Assay System (Promega) according to manufacturer's protocol. Chemiluminescence was measured by a Thermo luminometer (Labsystems).

Cytotoxicity. The Huh7-luc/neo cells were passaged into a 96-well plate. After incubation for 24 h (40–50 % confluence), the studied compounds at different concentrations were added to the cell culture. After three days of incubation at 37°C and 5% CO_2 (monolayer in the control probe was 100%), the cell viability was evaluated by the MTT test according to manufacturer's protocol (Sigma-Aldrich).

Western-blot. Proteins from cell lysates were separated by electrophoresis in 10% PAAG containing 0.1% SDS and were transferred on nitrocellulose membrane by the electromigration method. The membrane was treated with 5% nonfat dry milk (Bio-Rad) in PBST (phosphate saline buffer, pH 7.4, containing 0.1% Tween-20) for 60 min at room temperature. Primary antibodies to acetylated lysine (ab80178, Abcam) at 1 : 1000 dilution and antibodies to tubulin (Sigma) at 1 : 10000 dilution were added to membranes, which were incubated at 4°C overnight, followed by washing with PBST. The conjugate of horseradish peroxidase with specific secondary antibodies (antimouse, antirabbit, Santa Cruz) was added at 1 : 10000 dilution and incubated for 50 min at room temperature. Membranes were washed with PBST, and the signal was visualized using the ECL kit (Pierce-Thermo Scientific) and a High performance ECL film (GE Healthcare).

ACKNOWLEDGMENTS

The work was supported by a grant of the Russian Scientific Foundation no. 14-50-00060.

REFERENCES

1. Afdhal, N., Zeuzem, S., Kwo, P., Chojkier, M., Gitlin, N., Puoti, M., Romero-Gomez, M., Zarski, J.P., Agarwal, K., Buggisch, P., et al., *N. Engl. J. Med.*, 2014, vol. 370, pp. 1889–1898.
2. Reddy, K.R., Bourliere, M., Sulkowski, M., Omata, M., Zeuzem, S.S., Feld, J.J., Lawitz, E., Marcellin, P., Welzel, T.M., Hyland, R., et al., *Hepatology*, 2015. doi: 10.1002/hep.27826
3. Asselah, T. and Marcellin, P., *Liver Int.,* 2015, vol. 35, pp. 56–64.
4. Duggal, R., Patick, A.K., Zhao, W., Herlihy, K.J., Sha, E., and Liu, W., WO Patent no. 2004/073599 A2, 2004.
5. Spada, A.P., EU Patent no. 2144604 B1, 2011.
6. Sato, A., Saito, Y., Sugiyama, K., Sakasegawa, N., Muramatsu, T., Fukuda, S., Yoneya, M., Kimura, M., Ebinuma, H., Hibi, T., et al., *J. Cell. Biochem.*, 2013, vol. 114, pp. 1987–1996.
7. Kozlov, M.V., Kleymenova, A.A., Romanova, L.I., Konduktorov, K.A., Smirnova, O.A., Prasolov, V.S., and Kochetkov, S.N., *Bioorg. Med. Chem. Lett.,* 2013, vol. 23, pp. 5936–5940.
8. Kozlov, M.V., Kleymenova, A.A., Konduktorov, K.A., Malikova, A.Z., and Kochetkov, S.N., *Biochemistry* (Moscow), 2014, vol. 79, pp. 637–642.
9. Ai, T., Xu, Y., Qiu, L., Geraghty, R.J., and Chen, L., *J. Med. Chem.,* 2015, vol. 58, pp. 785–800.
10. Kozlov, M.V., Kleymenova, A.A., Romanova, L.I., Konduktorov, K.A., Kamarova, K.A., Smirnova, O.A., Prassolov, V.S., and Kochetkov, S.N., *Bioorg. Med. Chem. Lett.*, 2015, vol. 25, pp. 2382–2385.
11. Stevens, C.L. and Sherr, A.E., *Org. Chem.*, 1952, vol. 17, pp. 1177–1181.
12. Wagner, F.F., Olson, D.E., Gale, J.P., Kaya, T., Weïwer, M., Aidoud, N., Thomas, M., Davoine, E.L., Lemercier, B.C., Zhang, Y.L., et al., *J. Med. Chem.*, 2013, vol. 56, pp. 1772–1776.
13. Zhang, Y., Li, N., Caron, C., Matthias, G., Hess, D., Khochbin, S., and Matthias, P., *EMBO J.,* 2003, vol. 22, pp. 1168–1179.
14. Hendriks, B.S., Seidl, K.M., and Chabot, J.R., *BMC Syst. Biol.*, 2010. doi: 10.1186/1752-0509-4-23
15. Duggan, K.C., Hermanson, D.J., Musee, J., Prusakiewicz, J.J., Scheib, J.L., Carter, B.D., Banerjee, S., Oates, J.A., and Marnett, L.J., *Nat. Chem. Biol.*, 2011, vol. 7, pp. 803–809.
16. Busschots, K., Lopez-Garcia, L.A., Lammi, C., Stroba, A., Zeuzem, S., Piiper, A., Alzari, P.M., Neimanis, S., and Arencibia, J.M., Engel, M., et al., *Chem. Biol.*, 2012, vol. 19, pp. 1152–1163.

Translated by A. Levina