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2-[2-(Phenylcarbamoyl)hydrazinylidene]propanoates: synthesis, structure and *in vitro* study of the activity against influenza virus

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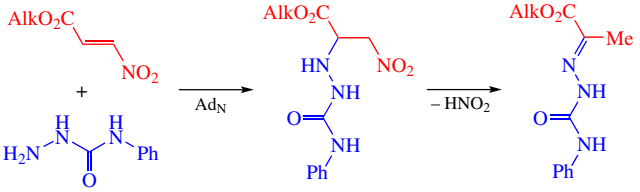
Alkyl (2*E*)-2-[2-(phenylcarbamoyl)hydrazinylidene]-propanoates were prepared from 3-nitroacrylates and *N*-phenylhydrazinecarboxamide. Their fine structure was confirmed by NMR and X-ray data. Cytotoxicity and antiviral activity against the A/Puerto Rico/8/34 (H1N1) influenza virus strain in MDCK cell culture were determined for the obtained compounds.

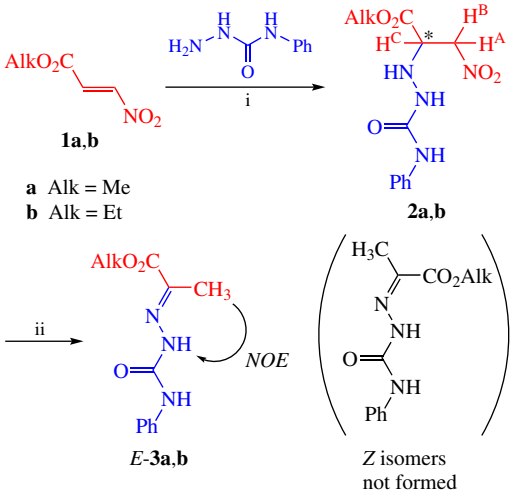


Keywords: nitroacrylates, *N*-phenylhydrazinecarboxamide, nucleophilic addition, aza-Michael adduct, hydrazone, influenza A (H1N1) virus, biological activity.

Semicarbazones of carbonyl compounds and their substituted analogues, easily accessible substances,[1,2] have practically valuable properties and exhibit various types of biological activity.[3,4] They are capable of forming complexes with transition metals, which is employed in analytical chemistry.[5,6] The existence of semicarbazones as two geometric isomers relative to the C=N bond makes them interesting models for theoretical organic chemistry.[7] The ability of one of the geometric forms of semicarbazones of keto carboxylic acids esters to heterocyclize creates conditions for their use in synthetic organic chemistry.[8–10]

In this study, we propose a two-step synthesis of *N*-phenylcarbamoylhydrazones of alkyl pyruvates based on the reaction of alkyl 3-nitroacrylates **1a**,**b** with *N*-phenylhydrazinecarboxamide (Scheme 1). It includes, at the first stage, the preparation of aza-Michael adducts, alkyl 3-nitro-2-[2-(phenylcarbamoyl)-hydrazinyl]propanoates **2a**,**b** in 77–78% yields by reacting 3-nitroacrylates **1a**,**b** with *N*-phenylhydrazinecarboxamide in glacial acetic acid at room temperature for 3 h.† The second stage, elimination of nitrous acid from the resulting aza adducts **2a**,**b**, proceeds at room temperature in an aqueous-alcohol solution in the presence of an equimolar amount of potassium hydroxide to afford products **3a**,**b**.‡ It should be noted that structurally comparable substituted hydrazones were previously obtained similarly by reacting 3-nitroacrylates with (nitroamino)-guanidine or (het)arenecarbohydrazides.[11]

According to ^{1}H and ^{13}C{^{1}H} NMR spectroscopy, the synthesized *N*-phenylcarbohydrazones of alkylpyruvates **3a**,**b** are stereohomogeneous. The results of studying compounds **3a**,**b** using ^{1}H–^{1}H NOESY NMR method reveal the presence of a nuclear Overhauser effect (NOE) between the protons for CH_3



Scheme 1 *Reagents and conditions*: i, AcOH, 18–20 °C, 3 h; ii, KOH, EtOH–H_2O (2 : 1), 18–20 °C, 3 h.

† *General procedure for the synthesis of alkyl 3-nitro-2-[2-(phenylcarbamoyl)hydrazinyl]propanoates* **2**. A solution of the corresponding nitroacrylate (2.3 mmol) in glacial acetic acid (5 ml) was added dropwise to a solution of *N*-phenylhydrazinecarboxamide (2.3 mmol) in glacial acetic acid (5 ml). The resulting mixture was stirred at 18–20 °C for 3 h. After removing the solvent, the residue was crystallized with a methanol. Product **2** was isolated as a colorless powder.

‡ *General procedure for the synthesis of alkyl (2E)-2-[2-(phenylcarbamoyl)hydrazinylidene]propanoates* **3**. To a solution of the corresponding compound **2** (0.71 mmol) in ethanol (10 ml), a solution of potassium hydroxide (0.71 mmol) in water (5 ml) was added. The reaction mixture was stirred at 18–20 °C for 3 h, then evaporated to the minimum amount of solvent. The precipitate was filtered off and recrystallized to give product **3** as a colorless powder.

and NH groups, which indicates the existence of these compounds in DMSO-d_6 solution as *E*-isomers. A similar result in the formation of a stereohomogeneous *E*-isomer is observed in the case of semicarbazones of alkyl succinates,[1] glyoxylates[9] and pyruvates,[12] as well as substituted hydrazones of alkyl pyruvates.[11]

X-ray diffraction study of ethyl (2*E*)-2-[2-(phenylcarbamoyl)-hydrazinylidene]propanoate **3b** confirms the *E*-configuration of the C=N bond (Figure 1).[§] The molecule forms an intramolecular hydrogen bond N(12)–H···N(8), which probably determines its preferential formation. In addition, the molecules in the crystal form centrosymmetric dimers supported by intermolecular hydrogen bonds, and the dimers form a layered structure (Figure 2). The crystal contains solvate molecules of benzene and water being the mixed crystalline solvate. Since the conformation of the three independent molecules of compound **3b** is the same, Figure 1 shows one of them (see also Online Supplementary Materials).

The crystal packing of compound **3b** is primarily determined by the system of hydrogen bonds, the formation of which involves the amino groups of the N(9) and N(12) atoms of three independent molecules and all the hydrogen atoms of three water molecules (see Figure 2). The participation of the benzene molecule in any strong intermolecular interactions was not found. Thus, the stoichiometry of the crystal is **3b** · H_2O · 1/3 C_6H_6.

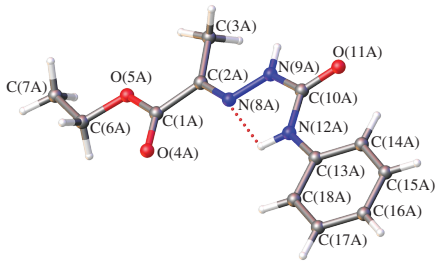


Figure 1 Geometry of one of the independent molecules of ethyl (2*E*)-2-[2-(phenylcarbamoyl)hydrazinylidene]propanoate **3b** in a crystal. The anisotropic displacement ellipsoids are shown at 50% probability level. The benzene solvate molecule and water molecules are not shown. The intramolecular hydrogen bond is shown by dotted line.

[§] *Crystal data for* **3b**. Crystals of **3b** solvate, namely, $3(C_{12}H_{15}N_3O_3)\cdot C_6H_6\cdot 3H_2O = C_{42}H_{57}N_9O_{12}$, $M = 879.97$, mp 143–145 °C, were obtained by slow evaporation of a benzene solution; triclinic. At 134 K, $a = 11.9472(13)$, $b = 14.1979(16)$ and $c = 14.4757(16)$ Å, $\alpha = 71.480(4)$, $\beta = 78.904(4)$ and $\gamma = 74.986(4)°$, $V = 2232.5(4)$ Å^3, $Z = 2$, $d_{calc} = 1.309$ g cm^{-3}, $\mu(MoK_\alpha) = 0.097$ mm^{-1}, space group $P\bar{1}$ (no. 2). The data were obtained on a Bruker D8 QUEST diffractometer (graphite monochromator, MoK_α radiation, $\lambda = 0.71073$ Å). The intensities of 91642 reflections were measured, 10683 of which were independent ($R_{int} = 0.1744$) and 5828 were observed with $I \geqslant 2\sigma(I)$. The recording ranges were: θ 1.5–28.0°, reflection indices: h –15: 15; k –18: 18; l –19: 19. Semi-empirical corrections for absorption were performed in the SADABS program.[13] The structure was solved by the direct method using the SHELXT program.[14] Non-hydrogen atoms were refined in isotropic and then in anisotropic approximation using the SHELXL program.[15] Hydrogen atoms were placed in the calculated positions and refined using the riding model. All calculations were performed using the WinGX[16] and APEX2[17] programs. Analysis of intermolecular contacts in the crystal and the drawings were performed using the PLATON[18] and MERCURY[19] programs. The final divergence factors were $R = 0.1314$, $R_w = 0.1836$ for all 10683 reflections, and $R = 0.0631$, R_w 0.1555 for the observed reflections with $I \geqslant 2\sigma(I)$. The goodness-on-fit was 1.050; the residual electron density extremums were –0.43 and 0.34 e Å^{-3}.

CCDC 2293177 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* http://www.ccdc.cam.ac.uk.

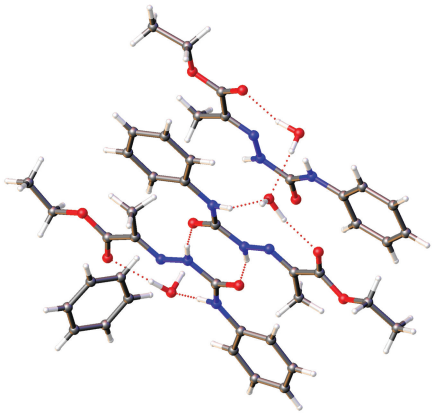


Figure 2 A fragment of **3b** crystal packing. The projection along axis *c*. Hydrogen bonds are shown by dotted lines.

Table 1 Antiviral properties of compounds **2** and **3** against influenza A (H1N1) virus in MDCK cell culture. Results of three independent experiments are presented as mean ± SD.

Compound	CC_{50}/μM	IC_{50}/μM	Selectivity index
2a	>1063	>1063	1
2b	>1013	>1013	1
3a	>1275	132 ± 19	10
3b	>1204	120 ± 14	10
Rimantadine	289 ± 12	58 ± 7	5

An *in vitro* study of the biological activity of compounds **2** and **3** was carried out in the Laboratory of Experimental Virology of Saint Petersburg Pasteur Research Institute of Epidemiology and Microbiology using MDCK (Madin-Darby canine kidney) cell culture against A/Puerto Rico/8/34 (H1N1) influenza virus (see also Online Supplementary Materials). Table 1 shows the values of the cytotoxic concentration (CC_{50}), inhibitory concentration (IC_{50}), and the selectivity index being the CC_{50}/IC_{50} ratio. The anti-influenza drug rimantadine, 1-(1-aminoethyl)adamantane, a blocker of the M2 protein of the influenza virus, was used as a reference. All the studied substances were non-toxic for MDCK cells even at the highest concentration used (300 μg ml^{-1}). In turn, alkyl (2*E*)-2-[2-(phenylcarbamoyl)hydrazinylidene]propanoates **3a**,**b** exhibited antiviral activity, and their virus inhibitory effect evaluated by selectivity index exceeded the corresponding value for the reference drug.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2024.02.031.

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