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New thiosemicarbazones possessing activity against SARS-CoV-2 and H1N1 influenza viruses

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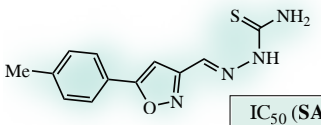
New thiosemicarbazones based on 5-arylisoxazole-3-carbaldehyde and substituted pyrrolecarbaldehydes have been obtained. The test for ability to inhibit the main viral protease 3Clpro of SARS-CoV-2 virus revealed a compound with activity against both corona- and influenza viruses.



IC_{50} (**SARS-CoV-2**) – 12.4 μM; SI – **30**
IC_{50} (**H1N1**) – 7.5 μM; SI – **32**

Keywords: thiosemicarbazones, isoxazoles, tetrahydroindoles, antiviral activity, protease 3Clpro, SARS-CoV-2 virus, influenza A (H1N1) virus, biological activity.

Thiosemicarbazones that can be easily obtained from ketones/aldehydes and thiosemicarbazide possess antiviral properties.[1] As early as 1950, the ability of *p*-aminobenzaldehyde-3-thiosemicarbazone to inhibit vaccinia viruses was identified.[2] Metisazone is one of the first synthetic antiviral drugs (Figure 1) developed for the treatment of orthopoxviruses, which acts by inhibiting mRNA synthesis.[3] Current studies in particular have suggested that metisazone and its derivatives may be inhibitors of the SARS-CoV-2 virus.[4] Importantly, agents with activity against a wide range of viruses have been found among thiosemicarbazones. For example, they can be inhibitors of MERS coronavirus entry,[5] inhibitors of Coxsackie B4 and respiratory syncytial virus,[6] while (+)-camphor-based thiosemicarbazone is an effective inhibitor of a wide range of orthopoxviruses, including smallpox virus.[7] Ambazone (*p*-benzoquinone amidinohydrazone thiosemicarbazone) is the active ingredient of Faringosept, an antiseptic for the oral cavity because it is active in a number of pathogens that trigger infections of the mouth and upper respiratory tract.[8] The high prospect of thiosemicarbazones as anticancer agents has been shown.[9,10]

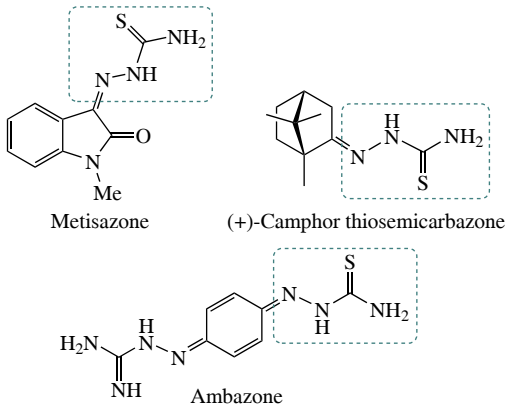
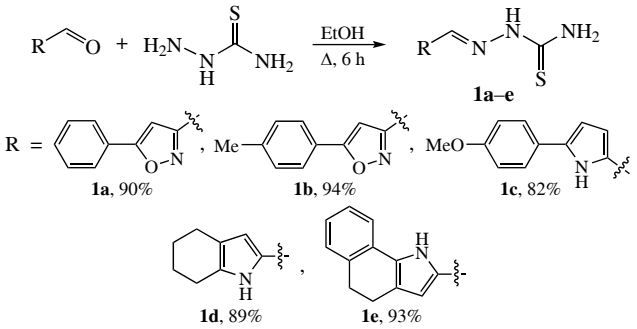


Figure 1 Structures of biologically active thiosemicarbazones.

It is known that 3-Chymotrypsin-like Protease of SARS-CoV-2 (3CLpro) is a successful target to the creation of potential antiviral agents.[11] Inhibition of the protease leads to inhibition of viral RNA replication. It is reported that the effect on this target is quite effective. An undoubted advantage of choosing this target is the fact that there are no homologues of 3CLpro in the human body.[12] This means that the effect on this protease will be associated with a minimum number of side effects, since potential inhibitors will act only on this protease.

In this work, we synthesized new thiosemicarbazones based on heterocyclic aldehydes and studied their antiviral properties. Heterocyclic compounds are widely present in nature, where they are involved in key biochemical processes and also form the basis of a large part of the medicinal compounds.[13] Compounds **1a**,**b** were synthesized from the corresponding 5-arylisoxazole-3-carbaldehyde (Scheme 1), the synthesis of the starting aldehydes was described earlier.[14,15] Substances **1a**,**b** are solid coloured compounds, yields from 90 to 94%. Compound **1c** was obtained starting from 5-(4-methoxyphenyl)-1*H*-pyrrole-2-carbaldehyde with a yield of 82%. In addition to isoxazole derivatives, new thiosemicarbazones **1d**,**e** were synthesized



Scheme 1

based on 4,5,6,7-tetrahydro-1*H*-indole-2-carbaldehyde and 4,5-dihydro-1*H*-benzo[*g*]indole-2-carbaldehyde, respectively, in 89 and 93% yields (see Scheme 1). The starting aldehydes were prepared according to the previously described method.[16]

The structure of the compounds was established on the basis of ^{1}H and ^{13}C NMR, IR and HR-MS analysis data. The structure of compound **1d** was also confirmed by X-ray diffraction analysis (Figure 2 and Table S1 of the Online Supplementary Materials).† Noteworthy, no hydrogen bonds are present in the crystal structure, and the molecules are linked by short N–H···S intermolecular contacts in a three-dimensional structure (the N···S distances are equal to 3.388, 3.415, and 3.727 Å for different non-equivalent contacts). The cyclohexane ring is disordered for two carbon atoms [C(7A), C(7B) and C(8A), C(8B)] with the site occupancy factors of 0.734(8) and 0.266(8).

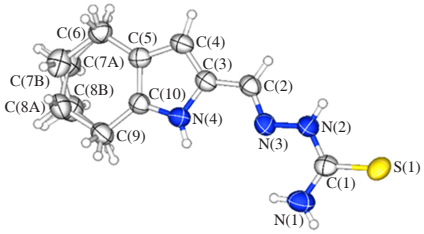


Figure 2 Solid-state structure of compound **1d**. Ellipsoids are shown at the 50% probability level. Atoms belonging to different conformations of the disordered cyclohexane ring are shown simultaneously.

† *Crystal data for* **1d**. $C_{10}H_{14}N_4S$ (M = 222.31), monoclinic, space group $C2/c$, a = 13.8705(10), b = 8.5289(4) and c = 19.6159(11) Å, β = 107.355(7)°, V = 2214.9(2) Å^3, Z = 8, T = 296(2) K, μ(MoKα) = 0.265 mm^{-1}, d_{calc} = 1.333 g cm^{-3}, 14007 reflections measured (4.35° ⩽ 2Θ ⩽ 52.744°), 2266 unique (R_{int} = 0.0553, R_{sigma} = 0.0350) which were used in all calculations. The final R_1 was 0.0340 [$I > 2\sigma(I)$] and wR_2 was 0.1014 (all data).

A single crystal was obtained by slow evaporation of a saturated solution of **1d** in ethanol at 296(2) K. Single-crystal X-ray diffraction data were collected at 296(2) K using a TD-5000 diffractometer [Mo-Kα radiation (λ = 0.71073 Å)] and a hybrid photon-counting detector. The investigations were performed using large-scale research facilities 'EXAFS spectroscopy beamline' at the Siberian synchrotron and terahertz radiation center. Data reduction was performed using the CrysAlisPro software.[23] The crystal structure was solved with the SHELXT program[24] using intrinsic phasing and refined with the SHELXL[25] refinement package using least squares minimisation with the Olex2 version 1.5 as a GUI.[26] All non-hydrogen atoms were refined anisotropically. The hydrogen atoms bonded to carbon atoms were placed in calculated positions and refined using a riding model with fixed isotropic displacement parameters [U_{iso}(H) = 1.2 U_{eq}(C)]. The hydrogen atoms bonded to N(1), N(2), and N(4) atoms were located from the Fourier difference map and refined isotropically without additional restraints. The disordered C(7A), C(7B) and C(8A), C(8B) atoms with the corresponding hydrogens were refined with SADI restraints.

CCDC 2372012 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* https://www.ccdc.cam.ac.uk.

Table 1 SARS-CoV-2 3CLpro-inhibiting activity of compounds **1a–e**.[a]

Compound	IC_{50}/μM	Compound	IC_{50}/μM
1a	65.25 ± 8.93	Disulfiram	6.25 ± 1.97
1b	84.98 ± 15.73	Ebselen	1.72 ± 0.4
1c	47.82 ± 6.17	GC376	0.023 ± 0.004
1d	6.90 ± 4.25	Nirmatrelvir	0.078 ± 0.014
1e	41.02 ± 12.53		

[a] The data presented are the mean of three independent experiments.

The search for new agents with activity against a wide range of viruses is an important task of organic and medicinal chemistry.[17–19] Herein, we tested the ability of compounds **1a–e** to inhibit the main viral protease 3Clpro of SARS-CoV-2 virus at the first stage. The testing was carried out using our original surrogate system with recombinant viral protein.[20] The results are given in Table 1, and known 3Clpro inhibitors were used as reference agents.

As can be seen from the presented data, the new substances exhibit activity against the main viral protease, and compound **1d** containing the tetrahydroindole fragment shows the highest activity. At the same time, the activity of the reference compounds is higher. In order to confirm the activity against SARS-CoV-2 virus, we further tested the new substances in a cytopathic test system using infectious SARS-CoV-2 virus strain Wuhan lineages Ba on Vero cell line (Table 2). Compounds **1a**, **1b** and **1d** did not show toxic properties against the cell line under study, thiosemicarbazones **1b** and **1e** exhibited the highest activity, but compound **1e** was quite toxic; however, no correlation with the data on activity against the main viral protease was observed. Compound **1b** has the highest selectivity index.

To identify the breadth of antiviral action, the new compounds were also tested as inhibitors of H1N1 influenza viruses. Influenza virus A/Puerto Rico/8/34 (H1N1) was propagated in MDCK cell line, rimantadine was used as a reference drug (Table 3).

To summarize, compounds **1b**, **1d**, **1e** do not show toxic properties against the MDCK cell line, while substances **1a**, **1c** are relatively toxic. The highest activity was found for thiosemicarbazones **1b** and **1c**. It is particularly important to note that agent **1b** proved to be non-toxic to both cell lines and exhibited inhibitory properties against both SARS-CoV-2 and H1N1 influenza viruses. Since this substance is weakly active against the major viral protease of coronavirus, we hypothesize that the mechanism of action is directed at other viral proteins. Previous studies show that thiosemicarbazones can exhibit antiviral activity by acting as inhibitors of RNA-dependent RNA polymerase.[21,22] Thus, we have synthesized new thiosemicarbazones and identified a compound with activity against both

Table 2 Antiviral activity of compounds **1a–e** against SARS-CoV-2 virus in Vero cells.[a]

Compound	CC_{50}/μM	IC_{50}/μM	SI
1a	406.5 ± 35.22	134.1 ± 15.5	2
1b	385.4 ± 33.12	12.4 ± 0.9	30
1c	88.7 ± 9.21	23.9 ± 3.1	4
1d	580.5 ± 41.11	137.3 ± 12.2	4
1e	37.7 ± 2.51	12.1 ± 2.1	3
Remdesivir	710.9 ± 21.25	3.8 ± 0.42	186

[a] Hereinafter CC_{50} is the cytotoxic concentration, which results in the death of 50% of cells. IC_{50} is the 50% virus-inhibiting concentration, which leads to 50% inhibition of virus replication. SI is the selectivity index, the ratio of CC_{50}/IC_{50}. The data presented are the mean of three independent experiments. The values for CC_{50} and IC_{50} are presented as the mean ± error of the experiment.

Table 3 Antiviral properties of compounds **1a–e** against influenza A (H1N1) virus in MDCK cell culture.

Compound	CC_{50}/μM	IC_{50}/μM	SI
1a	32.1 ± 2.6	15.0 ± 1.9	2
1b	245.3 ± 12.6	7.5 ± 0.9	32
1c	18.4 ± 0.9	3.6 ± 0.5	5
1d	1130.6 ± 59	150 ± 21	7
1e	585.1 ± 46	180 ± 23	3
Rimantadine	289 ± 12	58.2 ± 7.5	5

coronaviruses and influenza viruses. The mechanism of action of the hit compound will be further investigated.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.71267/mencom.7578.

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