The synthesis and antiviral activity against yellow fewer virus of 2-(4,6-di(pyrrolidin-1-yl)-1,3,5-triazin-2-yl)-N-(alkyl, aryl)hydrazine-1-carbothioamides

**Aim.** To synthesize 2-(4,6-di(pyrrolidin-1-yl)-1,3,5-triazin-2-yl)-N-(alkyl, aryl)hydrazine-1-carbothioamides and study their antiviral activity against yellow fever virus (YFV).

**Results and discussion.** The target 2-(4,6-di(pyrrolidin-1-yl)-1,3,5-triazin-2-yl)-N-(alkyl, aryl)hydrazine-1-carbothioamides were obtained in three-step format from cyanuric chloride in good to high yields. The carbothioamides synthesized were estimated to possess the antiviral activity against YFV. The results obtained indicate that most of the compounds studied show the inhibitory activity against YFV in concentrations ≤10 μg/mL. For the most active substances, EC_{50} was in the range of 0.06–2.2 μg/mL. Good effective concentration values were accompanied by low levels of cytotoxicity resulting in excellent selectivity index values. The data obtained also indicate that the presence of an alkyl substituent in ortho-position of the N-aryl fragment is crucial for an effective inhibition of YFV growth.

**Experimental part.** 2-(4,6-Di(pyrrolidin-1-yl)-1,3,5-triazin-2-yl)-N-(alkyl, aryl)hydrazine-1-carbothioamides were synthesized starting from cyanuric chloride in three steps by its successive interaction with two equivalents of pyrrolidine, hydrazine and a series of alkyl-arylisothiocyanates. The antiviral and cytotoxic activities of the target carbothioamides were studied in the Southern Research Institute (SRI, Birmingham, Alabama) by the viral cytotoxic effect reduction assay and the virus yield reduction assay.

**Conclusions.** 2-(4,6-Di(pyrrolidin-1-yl)-1,3,5-triazin-2-yl)-N-(alkyl, aryl)hydrazine-1-carbothioamides synthesized have been proven to be a promising class of compounds for treating such a severe viral disease as yellow fever.

**Key words:** 1,3,5-triazine; carbothioamides; yellow fever; antiviral activity; cytotoxicity
Yellow fever (yellow jack, yellow plague, bronze john) is a severe viral disease resulting from human infection with yellow fever virus (YFV) [1, 2]. It is endemic in tropical regions of Africa, as well as in Central and South America [3–5].

YFV is an RNA virus of the Flaviviridae family (genus Flavivirus). The virus persists in nature through transmission between non-human primates and mosquitoes fed upon them. The latter usually belong to Aedes species in Africa and Haemagogus species in the Americas. Humans may become infected with YFV through a sylvatic transmission vector when bitten by an infected mosquito (the so-called jungle yellow fever). However, inter-human transmission of the virus may also occur with Aedes aegypti as a vector of domestic transmission (the so-called urban yellow fever). In Africa, a third transmission vector is possible; it is known as the “intermediate cycle” involving both sylvatic and domestic vector species in inter-human transmission. From the epidemiological point of view, the biggest concern is given to the urban type of transmission as it can cause large-scale epidemics due to the high population density and low or no immunity to the disease [2].

One of the aspects of yellow fever danger lies in the field of its ability to spread rapidly causing the population damage. Due to the developed air and sea communication between the countries, there is a risk of importing infected mosquitoes from the endemic regions and their spread over new territories due to the ongoing climate change.

The clinical course of yellow fever proceeds through three phases, which are infection, remission, and intoxication. The first symptoms appear abruptly 2–9 days after YFV transmission to a living organism (infection phase). They include fever (up to 41 °C), headache, myalgia, malaise, nausea and vomiting. A short phase of remission may follow the infection phase, during which many patients (about 88%) recover. Other YFV-infected patients will progress to the intoxication phase, which is accompanied by fever, abdominal pain, nausea and vomiting, oliguria, jaundice, hepatic dysfunction, renal failure, hemorrhagic manifestations, encephalopathy [1].

According to the World Health Organization reports, about 200,000 cases of yellow fever are registered worldwide each year. The mortality rate among patients entering the phase of intoxication ranges from 20 to 50% in different outbreaks and case series [1].

Nowadays vaccination is the primary method used to prevent development of the disease [6]. Vaccines against YFV usually produce a strong and long-term immune response in all recipients [7–12]. Nevertheless, one should not consider vaccination as a totally successful and safe way of combating yellow fever. Thus, it can cause severe adverse events, including yellow fever vaccine-associated viscerotropic disease and acute neurotropic disease [13, 14] resulting in the immune system failure [15, 16].

There are no effective antiviral drugs for yellow fever by now. Meanwhile, several antiviral compounds are under investigation for use with this purpose, including sofosbuvir (Fig.) [17]. The study revealed that sofosbuvir may be used as an option to cure yellow fever until other more effective medicines are found and approved for human use.

During the high-throughput screening research aimed at finding novel antiviral substances, a benzodiazepine acetic acid derivative (BDAA, Fig.) with the promising antiviral properties was identified [18]. It comprises the 7-chloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one core, which is typical for many benzodiazepine drugs, and inhibits YFV potently.

Among other possible cures for yellow fever, one should note Favipiravir (sold under the brand name Avigan®) which is a pyrazinecarboxamide derivative (Fig.). The mechanism of its action is thought to be selective inhibition of viral RNA-dependent RNA polymerase [19].
Despite the availability of synthetic substances directly affected YFV, the symptomatic treatment is usually applied to people suffering from yellow fever. The treatment may consist of fluid replacement, hemodialysis (in case of kidney failure), blood transfusion (when indicated), vasopressors, antipyretics, antibiotics (if secondary infections appear) [20, 21].

Taking into account the information stated above modern medicine is in urgent need of effective, specific and safe antiviral drugs against yellow fever. Such a state of affairs encouraged us to carry out a research in this field. This paper describes the results of the synthesis and evaluation of the ant-YFV activity of some 1,3,5-triazine derivatives. The reason of our attention to the 1,3,5-triazine core is the previously published results revealing valuable pharmacological properties of its derivatives. Thus, 1,3,5-triazines have been found in a number of bioactive molecules, such as herbicides and pharmaceutical products [22]. Compounds comprising this heterocyclic system exhibit the cardiotonic [23], antitumor [24] and anticancer activities [25]. Moreover, antiviral agents of 1,3,5-triazine class have been proven to be effective against the human immunodeficiency viruses (HIV) [26, 27] and herpes simplex virus 1 (HSV-1) [28].

In the current research we set the task to synthesize a series of 2-(4,6-di(pyrrrolidin-1-yl)-1,3,5-triazin-2-yl)-N-(alkyl, aryl)hydrazine-1-carbothioamides 5, 7 (Scheme) and evaluate their usefulness in treating yellow fever. The starting compound in the synthetic strategy towards the target derivatives was cyanuric chloride (1). Its interaction with a double amount of pyrrrolidine in the acetone solution in the presence of potassium carbonate led to 2-chloro-4,6-di(pyrrrolidin-1-yl)-1,3,5-triazine (2) [29]. The latter easily gave 2-hydrazinyl-4,6-di(pyrrrolidin-1-yl)-1,3,5-triazine (3) when refluxed with hydrazine hydrate in propanol-2 [30]. Hydrazine derivative 3 was further used in the reaction with alkyl- 4a–c and arylisothiocyanates 6a–k producing the corresponding carbothioamides 5, 7 [31].

The next step was to study the antiviral activity of compounds 5a–c and 7a–k against YFV. These studies were performed in the Southern Research Institute (SRI, Birmingham, Alabama). Testing was done by the viral cytopathic effect (CPE) reduction assay and the virus yield reduction assay (Virus Yield). CPE was determined by the microscopic observation of cell culture monolayers (Visual), as well as the uptake of neutral red dye (Neutral Red). The results obtained are given in the Table below as EC\textsubscript{50}, EC\textsubscript{90} IC\textsubscript{50} and IS values (see notes under the Table).

The antiviral assay determining CPE is used for the initial screening of potential antiviral compounds. The antiviral activity of the compounds is evaluated based on their ability to prevent the virus from causing viral CPE in a cell culture. Compounds demonstrating the activity in the CPE assay are further evaluated by the virus yield reduction assay. This test evaluates the ability of the compounds to inhibit virus production in a cell culture.

The results obtained indicate that most of the compounds studied showed the inhibitory activity against YFV (EC\textsubscript{50}≤10 μg/mL) thought it was less pronounced as compared to the reference drug Infergen® (Interferon Alphacon) (Table).

\begin{table}
\centering
\begin{tabular}{|c|c|}
\hline
Drug & EC\textsubscript{50} (μg/mL) \\
\hline
Infergen® & \textless 3 \\
\hline
\end{tabular}
\caption{Inhibitory Activity of Infergen® and Compounds 5, 7 Against YFV}
\end{table}

Scheme. The synthesis of 2-(4,6-di(pyrrrolidin-1-yl)-1,3,5-triazin-2-yl)-N-(alkyl, aryl)hydrazine-1-carbothioamides 5a–c and 7a–k.
The antiviral activity of 2-(4,6-di(pyrrolidin-1-yl)-1,3,5-triazin-2-yl)-N-(alkyl, aryl)hydrazine-1-carbothioamide 5 and 7 against YFV

| Cmp | R               | Assay      | Trial* | EC_{50} | EC_{90} | IC_{50} | SI   |
|-----|-----------------|------------|--------|---------|---------|---------|------|
| 5a  | NHCH₃           | Neutral Red| 1      | >100    | –       | >100    | 0    |
| 5b  | NHC₃H₇          | Neutral Red| 1      | 31      | –       | 58      | 1.9  |
| 5c  | CH₃Ph           | Neutral Red| 1      | >36     | –       | 36      | 0    |
| 7a  | 2-Me-C₆H₄       | Visual     | 2      | 1.1     | –       | 23      | 22   |
| 7a  | 2-Me-C₆H₄       | Neutral Red| 1      | 3.2     | –       | 32      | 10   |
| 7a  | 2-Me-C₆H₄       | Neutral Red| 2      | 1.1     | –       | 25      | 23   |
| 7a  | 2-Me-C₆H₄       | Virus Yield| 3      | –       | 2.2     | –       | 11   |
| 7b  | 4-Me-C₆H₄       | Neutral Red| 1      | 11      | –       | 17      | 1.5  |
| 7c  | 2-MeO-C₆H₄      | Visual     | 2      | 5.1     | –       | 270     | 53   |
| 7c  | 2-MeO-C₆H₄      | Neutral Red| 1      | 10      | –       | >100    | >10  |
| 7c  | 2-MeO-C₆H₄      | Neutral Red| 2      | 2.8     | –       | 170     | 61   |
| 7c  | 2-MeO-C₆H₄      | Virus Yield| 3      | –       | 0.47    | –       | 362  |
| 7d  | 4-EtO-C₆H₄      | Neutral Red| 1      | 10      | –       | 29      | 2.9  |
| 7e  | 3-Cl-C₆H₄       | Neutral Red| 1      | >100    | –       | >100    | 0    |
| 7f  | 4-Cl-C₆H₄       | Neutral Red| 1      | 10      | –       | 28      | 2.8  |
| 7g  | 2,3-diMe-C₆H₄   | Visual     | 2      | 2.3     | –       | 18      | 7.8  |
| 7g  | 2,3-diMe-C₆H₄   | Neutral Red| 1      | 0.85    | –       | 7.9     | 9.3  |
| 7g  | 2,3-diMe-C₆H₄   | Neutral Red| 2      | 1.2     | –       | 18      | 15   |
| 7g  | 2,3-diMe-C₆H₄   | Virus Yield| 3      | –       | 0.09    | –       | 200  |
| 7h  | 2,4-diMe-C₆H₄   | Visual     | 2      | 1.6     | –       | 18      | 11   |
| 7h  | 2,4-diMe-C₆H₄   | Neutral Red| 1      | 3.2     | –       | 32      | 10   |
| 7h  | 2,4-diMe-C₆H₄   | Neutral Red| 2      | 1.4     | –       | 18      | 13   |
| 7h  | 2,4-diMe-C₆H₄   | Virus Yield| 3      | –       | 1.8     | –       | 10   |
| 7i  | 2,5-diMe-C₆H₄   | Visual     | 2      | 0.81    | –       | 32      | 40   |
| 7i  | 2,5-diMe-C₆H₄   | Neutral Red| 1      | 1.1     | –       | 10      | 9.1  |
| 7i  | 2,5-diMe-C₆H₄   | Neutral Red| 2      | 0.4     | –       | 18      | 45   |
| 7i  | 2,5-diMeC₆H₄    | Virus Yield| 3      | –       | 0.06    | –       | 300  |
| 7j  | 2,6-diMe-C₆H₄   | Neutral Red| 1      | 18      | –       | 89      | 4.9  |
| 7j  | 2,6-diMe-C₆H₄   | Visual     | 1      | 9       | –       | 16      | 1.8  |
| 7k  | 3,4-diMe-C₆H₄   | Neutral Red| 1      | 10      | –       | 18      | 1.8  |
| Infergen (Interferon Alphacon) | Neutral Red, Visual | –     | 0.01    | –       | –       | –    |

Notes: EC_{50} – the half maximal effective concentration causing 50% virus inactivation (μg/mL); EC_{90} – the effective concentration causing 90% virus inactivation (μg/mL); IC_{50} – 50% cytotoxicity value (μg/mL); SI – the selectivity index, it is the ratio that measures the window between cytotoxicity and the antiviral activity by dividing the given IC_{50} value into the EC_{50} or EC_{90} value.

* – Depending on the time the cell cultures are being infected the following cell concentrations were prepared: 100 000 cells/mL – 72 h incubation (3); 200 000 cells/mL – 48 h incubation (2); 400 000 cells/mL – 24 h incubation (1).

Considering the structure of the compounds under research one should note that N-alkyl substituted derivatives 5 demonstrated high values of EC_{50}, hence, were of interest. Meanwhile, compounds 7 bearing an N-aryl residue turned out to be much more potent against YFV. Their EC_{50} were in the range of 1–10 μg/mL in accordance with the CPE assay, except for 3-chloro-phenyl derivative 7e which was nearly inactive. The more precise virus yield reduction assay provided even less EC_{50} concentration values – 0.06–2.2 μg/mL. It is worth mentioning that good effective concentration values were accompanied by low levels of cytotoxicity resulting in the excellent selectivity index, which was up to 362. It is interesting that the substitution pattern of the N-aryl fragment significantly affected the antiviral potency of compounds 7. Thus, the most active molecules had a substituent in the ortho-position of the aryl residue. The introduction of the second substituent to the aryl residue altered the antiviral activity though it still remained to be high. However,
another ortho-position must be free as 2,6-dimethylphenyl derivative 7j lost in its antiviral efficacy as compared to other N-dimethylphenyl ones.

Thus, 2-(4,6-di(pyrrrolidin-1-yl)-1,3,5-triazin-2-yl)-N-(alkyl, aryl)hydrazine-1-carbothioamides 5 and 7 provide a high antiviral activity against YFV. The data obtained confirm the feasibility of further studies of the title compounds as potential antiviral agents.

**Experimental part**

**Chemistry part**

1H NMR spectra of compounds 5 and 7 were recorded on a Bruker VXR-400 spectrometer (Germany) operating at a frequency of 400 MHz in DMSO-d6 using tetramethylsilane (TMS) as an internal standard. Chemical shifts were reported in ppm using the δ scale. The melting points were measured on a small-sized heating table with an Electrothermal IA 9200 observation device. The elemental analysis was performed on an EuroEA 3000 elemental analyzer.

The general procedure for the synthesis of 2-(4,6-di(pyrrrolidin-1-yl)-1,3,5-triazin-2-yl)-N-(alkyl, aryl)hydrazine-1-carbothioamides 5a–c and 7a–k

2-(4,6-Di(pyrrrolidin-1-yl)-1,3,5-triazin-2-yl)-N-(p-tolyl)hydrazine-1-carbothioamide (7a).

Yield – 2.79 g (70%). M. p. 173–174°C (from ethanol). Anal. Calcd. for C22H19N5S: %: N 28.12; S 8.04. Found: % N 28.24; S 7.98. 1H NMR (400 MHz, DMSO-d6), δ, ppm: 8.39 (1H, s, NH); 8.84 (1H, s, NH); 9.21 (1H, s, NH); 9.36 (1H, s, NH).

7-(4-ethoxyphenyl)hydrazine-1-carbothioamide (7b).

Yield – 3.07 g (77%). M. p. 167–168°C (from propanol-2). Anal. Calcd. for C22H19N5S: %: N 27.91; S 7.96. 1H NMR (400 MHz, DMSO-d6), δ, ppm: 8.25–8.46 (8H, m, 2 × CH3CH=); 7.79 and 7.40 (4H, d, J = 8.1 Hz, CH=); 8.53 (1H, s, NH); 9.27 (1H, s, NH); 9.40 (1H, s, NH).

8-(4-methoxyphenyl)hydrazine-1-carbothioamide (7c).

Yield – 2.98 g (72%). M. p. 188–189°C (from propanol-2). Anal. Calcd. for C22H19N5S: %: N 27.33; S 7.87. 1H NMR (400 MHz, DMSO-d6), δ, ppm: 8.35–8.46 (8H, m, 2 × CH3CH=); 6.90–6.87 (4H, m, C4H3); 8.76 (1H, s, NH); 8.95 (1H, s, NH); 9.50 (1H, s, NH).

9-(4-ethoxyphenyl)hydrazine-1-carbothioamide (7d).

Yield – 3.47 g (81%). M. p. 161–162°C (from propanol-2). Anal. Calcd. for C22H19N5S: %: N 26.15; S 7.48. Found: % N 26.07; S 7.55. 1H NMR (400 MHz, DMSO-d6), δ, ppm: 1.31 (3H, t, J = 7.2 Hz, OCH3CH=); 1.82–1.86 (8H, m, 2 × CH3CH=); 3.42–3.46 (8H, m, 2 × CH3CH=); 4.00 (2H, q, J = 7.2 Hz, OCH3CH=); 6.84 and 7.31 (4H, d, J = 8.4 Hz, C4H3); 8.52 (1H, s, NH); 9.21 (1H, s, NH); 9.36 (1H, s, NH).

N-(3-chlorophenyl)-2-(4,6-di(pyrrrolidin-1-yl)-1,3,5-triazin-2-yl)hydrazine-1-carbothioamide (7e).

Yield – 3.69 g (88%). M. p. 221–222°C (from ethanol). Anal. Calcd. for C22H19ClN5S: %: N 26.75; S 7.65. Found: % N 26.54; S 7.59. 1H NMR (400 MHz, DMSO-d6), δ, ppm: 1.83–1.86 (8H, m, 2 × CH3CH=); 3.42–3.46 (8H, m, 2 × CH3CH=); 7.14–7.72 (4H, m, C4H3); 8.60 (1H, s, NH); 9.52 (1H, s, NH); 9.65 (1H, s, NH).

N-(4-chlorophenyl)-2-(4,6-di(pyrrrolidin-1-yl)-1,3,5-triazin-2-yl)hydrazine-1-carbothioamide (7f).

Yield – 3.81 g (91%). M. p. 206–207°C (from ethanol). Anal. Calcd. for C22H19ClN5S: %: N 26.75; S 7.65. Found: % N 26.9; S 7.70. 1H NMR (400 MHz, DMSO-d6), δ, ppm: 1.83–1.87 (8H, m, 2 × CH3CH=); 3.39–3.45 (8H, m, 2 × CH3CH=); 7.33 and 7.55 (4H, d, J = 8.8 Hz, C4H3); 8.54 (1H, s, NH); 9.41 (1H, s, NH); 9.59 (1H, s, NH).
2-(4,6-Di(pyrrrolidin-1-yl)-1,3,5-triazin-2-yl)-N-(2,3-dimethylphenyl)hydrazine-1-carbothioamide (7g)

Yield – 2.85 g (69%). M. p. 178–179°C (from ethanol). Anal. Calcld. for C₂₀H₂₀N₈S₈; %: N 27.16; S 7.77. Found; %: N 27.23; S 7.83. ¹H NMR (400 MHz, DMSO-d₆), δ, ppm: 1.83–1.87 (8H, m, 2 × CH₂CH₃); 2.04 (3H, s, CH₃); 2.23 (3H, s, CH₃); 3.42–3.47 (8H, m, 2 × CH₃CH₂); 6.93–7.05 (3H, m, C₆H₃); 8.56 (1H, s, NH); 9.23 (2H, br. s, 2NH).

2-(4,6-Di(pyrrrolidin-1-yl)-1,3,5-triazin-2-yl)-N-(2,4-dimethylphenyl)hydrazine-1-carbothioamide (7h)

Yield – 2.72 g (66%). M. p. 165–166°C (from propanol-2). Anal. Calcld. for C₂₀H₂₀N₈S₈; %: N 27.16; S 7.77. Found; %: N 27.01; S 7.89. ¹H NMR (400 MHz, DMSO-d₆), δ, ppm: 1.82–1.85 (8H, m, 2 × CH₂CH₃); 2.13 (3H, s, CH₃); 2.29 (3H, s, CH₃); 3.42–3.47 (8H, m, 2 × CH₃CH₂); 6.83–7.13 (3H, m, C₆H₃); 8.34 (1H, s, NH); 8.97 (2H, br. s, 2NH).

2-(4,6-Di(pyrrrolidin-1-yl)-1,3,5-triazin-2-yl)-N-(2,6-dimethylphenyl)hydrazine-1-carbothioamide (7i)

Yield – 3.30 g (80%). M. p. 175–176°C (from propanol-2). Anal. Calcld. for C₂₀H₂₀N₈S₈; %: N 27.16; S 7.77. Found; %: N 27.11; S 7.85. ¹H NMR (400 MHz, DMSO-d₆), δ, ppm: 1.83–1.87 (8H, m, 2 × CH₂CH₃); 2.10 (3H, s, CH₃); 2.24 (3H, s, CH₃); 3.42–3.47 (8H, m, 2 × CH₃CH₂); 6.93–7.06 (3H, m, C₆H₃); 8.57 (1H, s, NH); 9.17 (1H, s, NH); 9.24 (1H, s, NH).

2-(4,6-Di(pyrrrolidin-1-yl)-1,3,5-triazin-2-yl)-N-(2,5-dimethylphenyl)hydrazine-1-carbothioamide (7j)

Yield – 2.74 g (66%). M. p. 161–162°C (from propanol-2). Anal. Calcld. for C₂₀H₂₀N₈S₈; %: N 27.16; S 7.77. Found; %: N 27.27; S 7.91. ¹H NMR (400 MHz, DMSO-d₆), δ, ppm: 1.82–1.87 (8H, m, 2 × CH₂CH₃); 2.14 (6H, s, 2 × CH₃); 3.42–3.47 (8H, m, 2 × CH₃CH₂); 6.98–7.06 (3H, m, C₆H₃); 8.58 (1H, s, NH); 9.10 (1H, s, NH); 9.21 (1H, s, NH).

2-(4,6-Di(pyrrrolidin-1-yl)-1,3,5-triazin-2-yl)-N-(2,3-dimethylphenyl)hydrazine-1-carbothioamide (7k)

Yield – 2.72 g (73%). M. p. 174–175°C (from propanol-2). Anal. Calcld. for C₂₀H₂₀N₈S₈; %: N 27.16; S 7.83. Found; %: N 26.98; S 7.65. ¹H NMR (400 MHz, DMSO-d₆), δ, ppm: 1.83–1.86 (8H, m, 2 × CH₂CH₃); 2.18 (6H, s, 2 × CH₃); 3.41–3.46 (8H, m, 2 × CH₃CH₂); 7.03–7.22 (3H, m, C₆H₃); 8.52 (1H, s, NH); 9.23 (1H, s, NH); 9.30 (1H, s, NH).

Pharmacological part

The viral cytopathic effect (CPE) reduction assay and the virus yield reduction assay were performed according to the known procedures [32, 33]. Briefly, the viral cytopathic effect (CPE) reduction assay consists in the following. Vero cells were seeded into 96-well clear plates, and all of the plates were incubated for about 18 h prior to use for assays. Compounds were first applied to the cell monolayers, followed within minutes by addition of virus suspensions. Uninfected wells with the compounds tested were used for assessing cytotoxicity (IC₅₀), infected wells – for assessing the antiviral activity, drug-free infected wells – as virus controls, uninfected drug-free wells – as cell controls, background wells containing only water. After incubation the microplates were read visually to estimate the percentage of cell destruction caused by the virus infection or by cytotoxicity. When the maximum CPE was observed, each plate was treated with a neutral red dye followed by incubation of the plates at 37°C. The neutral red solution used was prepared by dilution of its 0.68% solution with the physiological saline solution in the ratio of 1:20. The resulting solution (0.1 mL) was added to each cell monolayer. The plates were incubated for 2 h in order to allow the dye to be adsorbed. After incubation the plates were aspirated dry, and the monolayers were washed twice with a brine/phosphate buffer mixture followed by addition of 0.25 mL of 1:1 Sörensen’s citrate buffer (pH 4.2) in ethanol to each well to desorb the dye. To complete desorption of the dye from the cells the plates were placed in a dark place for 30 min at room temperature and after read using the colorimetry method at 540 nm. Absorbance units were converted to percentages of uninfected control cells. Fifty percent virus inhibitory (EC₅₀) or 50% cytotoxicity (IC₅₀) values were determined by linear regression using an Excel spreadsheet.

The virus yield reduction assay is a two-step assay where the virus is first produced in Vero cells cultures containing the antiviral substance in varying dilutions, followed later by titration of the samples for the virus titer by the endpoint dilution in 96-well plates. Dilutions of the test compound were assayed, and the effective antiviral concentration was determined by the regression analysis.

Vero cells were infected with the virus at 0.3 pfu/cell and then incubated for 24 h. After incubation the infected cells were exposed to solutions of the test compounds for 72 h. After virus adsorption the culture fluid was diluted to the required concentration by semilogarithmic dilutions of test compounds prepared in the assay medium. Depending on the situation, the supernatants were pooled either 24 h or 72 h after infection, and the extracellular viral infectivity titers were determined in duplicate by analysis of plaques in Vero cell monolayers. The endpoint for the 24-hour assay is the effective concentration (EC₅₀), which reduces the virus yield by 50% compared to control cultures. The effective concentration (EC₅₀) was determined from the data obtained.

Conclusions

2-(4,6-Di(pyrrrolidin-1-yl)-1,3,5-triazin-2-yl)-N-(alkyl, aryl)hydrazine-1-carbothioamides synthesized have been proven to be a promising class of compounds for treating such a severe viral disease as yellow fever.

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26. Kukla, M. J.; Heeres, J.; Janssen, P. A. J.; Ludovici, D. W.; Moereels, H. E. L. (Janssen Pharmaceutica NV). Substituted diamino-1,3,5-triazine derivatives. Eur. Patent EP0834507A1, Apr 08, 1998.

27. De Corte, B.; De Jonge, M. R.; Heeres, J.; Janssen, P. A. J.; Kavash, R. W.; Koymans, L. M. H.; Kulda, M. J.; Ludovici, D. W.; Van Aken, K. J. A. 2,4-Disubstituted triazine derivatives. Int. Patent WO0027828A2, May 18, 2000.

28. Mibu, N.; Yokomizo, K.; Yuzuriha, A.; Otsubo, M.; Kawaguchi, Y.; Sano, M.; Sakai, I.; Nakayama, K.; Zhou, J.-R.; Sumoto, K. Antiviral Activities of Some New 2,4,6-Trisubstituted 1,3,5-Triazines Having Alkoxy and/or Alkylamino Groups. Heterocycle 2017, 94 (9), 1653–1677. https://doi.org/10.3987/COM-17-13735.

29. Yaguchi, Sh.; Koshimizu, I.; Yoshimi, H.; Matsuno, T.; Watanabe, T.; Tsuchida, Y.; Saitoh, K. (Zenyaku Kogyo Kabushiki Kaisha). Treatment of prostate cancer, melanoma or hepatic cancer. US Pat. 2007244110A1, Oct 18, 2007.

30. CIBA LTD. New Triazines and process for preparing same. Pat. GB 942961, Nov 27, 1963.

31. Demchenko, A. M.; Barchina, O. I.; Sukhoveev, V. B.; Smol’skyi, O. C.; Kuрач, A. V. Синтез та антиоксидантні властивості похідних 2-R-{4,6-диметилнітрил-1-йді-}[1,3,5]-триазин-2-йді-N-гідразинкарботіоамідів. Наукові записки Тернопільського національного педагогічного університету ім. Володимира Гнатюка. Сер. Хімія 2011, 18, 13–19.

32. Smee, D. F.; Hurst, B. L.; Evans, W. J.; Clyde, N.; Wright, S.; Peterson, C.; Jung, K.-H.; Day, C. W. Evaluation of cell viability dyes in antiviral assays with RNA viruses that exhibit different cytopathogenic properties. Journal of Virological Methods 2017, 246, 51–57. https://doi.org/10.1016/j.jviromet.2017.03.012.

33. Bacon, T. H.; Howard, B. A.; Spender, L. C.; Boyd, M. R. Activity of penciclovir in antiviral assays against herpes simplex virus. J. Antimicrob. Chemother. 1996, 37 (2), 303–313. https://doi.org/10.1093/jac/37.2.303.

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