Antiviral activity of 1-(1-arylimidazolidine-2-ylidene)-3-(4-chlorobenzyl)urea derivatives

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Abstract

The aim of the study was evaluation of the biological activity of 1-(1-arylimidazolidine-2-ylidene)-3-(4-chlorobenzyl)urea derivatives (**I – IV**) against the Coxsackievirus B3 (CVB3).

The cytotoxic activity was tested in HEK293 cell culture. Cells were incubated for 72 hours at 37°C in a 5% CO_2 atmosphere. Cytotoxicity of substances was measured by the MTT test. After determining the highest non-toxic concentration of substances their activity against the Coxsackievirus B3 from the *Picornaviridae* family was estimated. In order to check the antiviral activity of the compounds, a virus suspension was added to the cell culture and incubated for 1 hour at 37 °C. The virus was then removed from the culture and the tested compounds were added. The cell culture was incubated at 37°C in a 5% CO_2 atmosphere until the cytophatic effect in the virus control was achieved, and then the virus was titrated. The virus titre was calculated by the Reed-Muench method. EC_{50} values of compounds **I – IV** were contained within the range of 211.4 – 359.7 µg/ml. The research demonstrated that only compound **II** slightly influenced the CVB3 replication by reducing the virus replication level by 0.77 log, which resulted in reducing the titre by 12.8%.

Key words

1-(1-arylimidazolidine-2-ylidene)-3-(4-chlorobenzyl)urea derivatives, HEK293 cells, antiviral activity, Coxsackievirus B3

INTRODUCTION

The increasing danger of viral infections generates the need to search for new antiviral drugs which are non-toxic for human beings. There are two trends in the research aimed at discovering potential antiviral activity factors. One trend deals with the synthesis of new derivatives of already existing antiviral drugs; the chemical synthesis of such compounds is oriented towards creating a new, more effective drug. The other trend of working on new antiviral drugs, looking for natural compounds, mainly of plant origin, in order to obtain potential chemotherapeutics [1].

In order to examine the antiviral activity of new substances, their influence on the propagation of various experimental models of RNA viruses with the simultaneous evaluation of the cytotoxicity of these preparations, is defined.

Coxsackieviruses are important human pathogens, causing a remarkable variety of diseases, from minor common colds to fatal myocarditis and neurological disorders. Coxsackievirus B3 (CVB3) is a member of the genus *Enterovirus* of the *Picornaviridae* family that contains a single-stranded, positive-sense RNA genome. It is a cardiotropic virus known to induce viral myocarditis [2] in children and young people, which is a common cause of acute heart failure and dilated cardiomyopathy [3, 4].

In recent years, a number of simple and fused derivatives of imidazoline have been synthesized at the Department of Synthesis and Technology of Pharmaceutical Substances. In many cases they exhibited different activities.

In the course of searching for new compounds with potential pharmacological activity 1-(1-arylimidazolidine-2-ylidene)-3-arylalkylurea derivatives were received.

The presented study evaluates the antiviral activity of 1-(1-arylimidazolidine-2-ylidene)-3-arylalkylurea derivatives against the Coxsackievirus B3 (CVB3).

MATERIALS AND METHOD

Compounds. The synthesis of compounds (**I – IV**) is shown in Figure 1. In the reaction of ethyl N-(1-arylimidazolidine-2-ylidene)carbamic acid ester with 4-chlorbenzylamine derivatives of 1-(1-arylimidazolidine-2-ylidene)-3-arylalkylurea were obtained.

1-(1-arylimidazolidine-2-ylidene)-3-(4-chlorbenzyl)urea (I – IV) (general procedure). Ethyl N-(1-arylimidazolidine-2-ylidene)carbamic acid ester (0.02 mol) and 0.02 mol (2.8g) 4-chlorbenzylamine were solved in 50 ml of methanol and refluxed for 6 h. The solvent was removed. The precipitate was filtered off and crystallized from propan-2-ol.

Chemistry. Melting points were determined on a Böetius apparatus and are given uncorrected. The ¹H NMR spectra were recorded on a Bruker Avance 300 in DMSO-d₆ with TMS as an internal standard. Chemicals were purchased from Aldrich or Merck and used without further purification. The purity of obtained compounds was checked by TLC on Merck plates $SiO_2\ F_{234}$ in a $CHCl_3/CH_3OH$ (10:1) solvent system with UV visualization. Elemental analyses were performed on a Perkin-Elmer analyser and were in the range of $^+_-$ 0.535 for each analyzed element (C, H, N, Cl).

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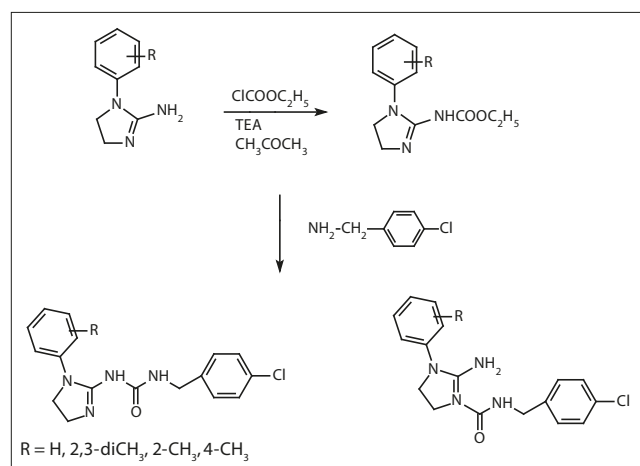
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Figure 1. Synthesis of 1-(1-arylimidazolidine-2-ylidene)-3-arylalkylurea derivatives (**I–IV**)

Cell cultures and viruses. HEK293 cell culture (human embryonic kidney) from the American Type Culture Collection (ATCC No. CRL – 1573) was used in the experiment. The media in the culture (Minimum Essential Medium Eagle, Sigma) were supplemented with 10% foetal bovine serum (FBS, Sigma), 100 U/ml of penicillin and 0.1 mg/ml of streptomycin (Polfa-Tarchomin, Poland). The cell culture was incubated at 37 °C in a 5% CO_2 atmosphere.

For antiviral activity of examined compounds the Coxsackievirus B3 – CVB3 – (ATCC No. VR-30) from the American Type Culture Collection was used. The virus was propagated in the HEK293 cell culture. Virus stock was stored at -70 °C until used.

Cytotoxicity assay. Compounds were dissolved in dimethyl sulfoxide (DMSO – POCH, Poland) in the concentration of 50 mg/ml and further diluted with a complete test medium. 100 μl of the HEK293 cell culture prepared were plated into 96-well plastic plates (NUNC) at a cell density 2×10^4 cells per well. After 24 h incubation at 37°C the media were removed and the cells were treated with a solution of the examined substance diluted in the media with 2% of serum. The cells were submitted to a series of compound concentrations, from 1,000 μg/ml to 1.9 μg/ml. Two-fold serial dilutions of compounds were added to the cells in triplicates. The culture cells were incubated for 72 h at 37°C in the 5% CO_2 atmosphere.

Cytotoxicity of tested compounds was estimated with the use of the MTT method, described by Takenouchi and Munekata [5]. The MTT method is a quantitative colorimetric toxicity test, based on the transformation of yellow, soluble tetrazolium salts (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) to purple-blue insoluble formasane. This process occurs naturally in the mitochondria of living cells. After 72 h incubation with compounds, the cell cultures were supplemented with 10 μl per well of 5 mg/ml MTT (Sigma) stock in PBS (BIOMED, Poland), and the incubation continued for 4 h at 37⁰C. Then, 100 μl of aqueous solution containing 50% dimethylformamide (POCH, Poland) and 20% SDS (Sigma) to solubilise the insoluble formasane precipitates produced by MTT was added. After all-night incubation, the absorbance was measured by plate reader (Epoch, BioTek) at two wavelengths – 540 and 620 nm. On the basis of the results, the cytotoxic concentration (EC_{50}),

which is the amount of tested substance required to reduce the number of viable cells by 50% compared to the control culture, was determined and calculated by using Gen 5 2.01.14 USA, BioTek software. The investigation was carried out twice.

Antiviral activity assay. After 24 h incubation, the cell culture was infected with a virus in the dose of 100 $TCID_{50}$/ml. After 1 h incubation at 37 °C the suspension of the virus was removed and the media with 2% of serum together with the tested compounds in the maximum nontoxic concentration were added to the cell cultures. The virus diluted in the culture media without tested compounds was used as a control. Ribavirin was used as a reference compound. After 48 h incubation at 37 °C, the cells were frozen and after thawing the virus was titrated in the HEK293 cell culture. The cytopathic effect (CPE) of the virus was examined by a light microscope and the titre of virus was estimated according to the Reed-Muench method [6]. Viral titers were determined by tissue culture infection dose ($TCID_{50}$) assays.

RESULTS AND DISCUSSION

The influence of 1-(1-arylimidazolidine-2-ylidene)-3-(4-chlorobenzyl)urea derivatives on the HEK293 cell culture after incubation for 72 h is presented in Table 1.

Table 1. Cytotoxicity of compounds **I–IV**

Compounds	Cell line/EC_{50} (μg/ml)[a]
	HEK293
I	359.7 ± 17.3
II	225.0 ± 21.2
III	211.4 ± 3.2
IV	232.7 ± 19.8
ribavirin	> 5000

[a]Mean ± S.D. values come from two independent experiments
EC_{50} is the cytotoxic concentration required to reduce the number of viable cells by 50%

DMSO used as an eluent for the examined compounds in tested concentration had no toxic effect on cell cultures. All compounds were evaluated for their cytotoxicity on cell line by a standard MTT assay.

EC_{50} values of compounds **I – IV** were contained within the range of 211.4 – 359.7 μg/ml. Compounds were tested for *in vitro* antiviral activity against Coxsackievirus B3 (CVB3) using the cytopathic effect (CPE) inhibitory assay. The result is presented in Table 2.

Table 2. Antiviral activity of the compounds against CVB3

Compounds	MNCC [μg /ml][b]	CVB3 [$TCID_{50}$ /ml][c]
I	125	5.67 ± 0.37
II	125	5.23 ± 0.49
III	125	6.00 ± 0.53
IV	125	6.00 ± 0.53
control	0	6.00 ± 0.53
ribavirin	5000	2.00 ± 0.53

[b]MNCC Maximum noncytotoxic concentration
[c] Virus titres are shown in log: Mean ± S.D.

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Noncytotoxic concentration of 125 µg /ml was used for testing the antiviral activity of all compounds, except for ribavirin where the concentration was 5,000 µg /ml. The research demonstrated that only compound **II** slightly influenced the CVB3 replication by reducing the virus replication level by 0.77 log, which resulted in reducing the titre by 12.8%.

The presented study is a preliminary report concerning research on the antiviral activity of 1-(1-arylimidazolidine-2-ylidene)-3-(4-chlorobenzyl) urea derivatives. This is a new group of compounds of biological activity not yet extensively described in the literature.

The activity of 4-vinyl-1-arylsulfonylimidazolidinones was reported as an anticancer agent against the various cancer cell lines (human colon carcinoma, human chronic myelogenous leukemia, human ovarian adenocarcinoma and human lung carcinoma). Some of them demonstrated this activity [7].

N-[2-(diethylenediamidophosphonyl)vinyl]-N'-arylurea derivatives demonstrated antitumour activity [8].

Research by Agadzhanyan et al. proved the hypoglycaemic activity of N-arylsulfonyl-N'-[7-(1,3,5-triazaadamantyl)] urea [9].

Research on the influence of 1-aryl-6-benzylimidazo [1, 2-a] [1, 3, 5] triazines on the central nervous system of mice in some behavioural tests proved that these derivatives show CSN depressive activity. Presented derivatives exerted significant antinociceptive activity, it can be found that this analgesia may be the result of interaction with opioid receptors [10].

Despite the impact of CVB3 infections on human health, there is no drug approved for the treatment of these infections. Currently, a number of compounds have been described as selective inhibitors of CVB3 replication *in vitro* [11]. Aguado et al. recently synthesized a number of 9-arylpurines with significant activity against CVB3. The most active compounds inhibited CVB3 replication cycle in a dose dependant manner with EC_{50} values between 5 – 8 µM. Moreover, those compounds showed low toxicity on tested cell lines[12]. Compounds with antiviral activity towards CVB3 were also identified in plants. Xu et al. isolated a new A-type trimeric proanthocyanidins with two doubly-bonded interflavanoid linkages – litchitannin A2 from lychee (*Litchi chinensis* Sonn. cv. Heiye) seeds. Litchitannin A2 displayed *in vitro* anti-CVB3 activity with IC_{50} and TI (CC_{50}/IC_{50}) values of 35.2 µg/mL and 3.2, which was comparable to virazole, of which IC_{50} and TI values were 69.2 µg/mL and 6.2 [13]. Despite of those discoveries there is still need of further research on new antiviral compounds.

The presented activity of 1-(1-arylimidazolidine-2-ylidene)-3-(4-chlorobenzyl) urea derivatives is not significant and there is still a need for further research on antiviral activity of other urea derivatives. Future studies will involve the virucidal activity of those compounds on CVB3, as well as the influence on various steps of the replication cycle. Furthermore, DNA viruses will be incorporated into the next phase of the research.

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