ANTI VIRAL ACTIVITY OF A NOVEL POLYNITROGENATED HETEROTRICYCLIC SYSTEM DERATIVE

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Abstract

The inhibitory action of the selected derivative of a novel heterocyclic ring system of imidazo[1,2-d][1,2,4]triazolo[4,3-b][1,2,4]triazole, i.e. 6-(2,3-dimethylphenyl)-7,8-dihydro-6H-imidazo[1,2-d][1,2,4]triazolo[4,3-b][1,2,4]triazole-3(2H)-thione on human adenovirus 5 (Ad-5) replication and its cytotoxicity for GMK cells were presented. The conducted experiments revealed that the examined compound possesses virucidal activity against Ad-5. The investigated heterocycle inhibited viral replication and decreased the final virus yield by 0.56 log, which corresponds to the percentage level of inhibition 17.3%, independently from the used concentration. The cytotoxicity of the investigated derivative was dependent on dose and time of incubation.

Key words: human adenovirus 5, imidazo-triazolo-triazole-thione, virucidal activity, cytotoxicity, in vitro experimentation.

Imidazoline (4,5-dihydrimidazol) and its derivatives occupy a pivotal position in modern medicinal chemistry. Many of them demonstrate pharmacological activity as ligands of the imidazoline receptor. Imidazoline ring is the structural element of many drugs possessing different pharmacological activities. The following adrenergic imidazoline derivatives are applicable in medicine: naphazoline, xylometazoline, oxymetazoline, fenoxazoline, tetrazyzoline. The others: tolazoline and phenolamine are used as α-adrenolytics, cifenline as antiarrhythmic, clonidine as hypotensive and antazoline as antihistaminic (10, 14).

Furthermore, from the literature data it follows, that depending on the type of substituent certain derivatives of 1,2,4-triazole show antifungal (27), fungicidal (7), insecticidal (26), antimicrobial (3, 16, 21), antiviral (1, 15, 28) and antitumour (1, 3, 4, 8) properties. Furthermore, several compounds containing the 1,2,4-triazole moiety were reported as anticonvulsants (9), antidepressants (2) and plant growth regulants (6). Recently, there is a widespread interest in the design of novel 1,2,4-triazole derivatives because of their potential anticancer activity associated with their skeleton (1, 3, 4, 8).

Prompted by these reports, and in continuation of search for polyheterocyclic bioactive molecules (17, 19, 20, 22) it seemed worthwhile to evaluate the biological importance of the derivative of a novel ring system, which contain three fused heterocyclic rings: one imidazolidine and two 1,2,4-triazole moieties. In this paper we would like to present the biological activity assessment for respective 6-(2,3-dimethylphenyl)-7,8-dihydro-6H-imidazo[1,2-d][1,2,4]triazolo[4,3-b][1,2,4]triazole-3(2H)-thione. The examined compound was obtained by cyclcondensation of 7-(2,3-dimethylphenyl)-3-hydrazino-5H-6,7-dihydrimidazo[2,1-c][1,2,4]triazole with carbon disulfide according to bisantrene, therapeutically used against adult acute non-lymphotic leukaemia is structurally based on the 2-hydrazino-Δ3-imidazoline heterocyclic system (10).
the procedure reported in the previous paper (23). IR, \(^1\)H NMR, EI-MS spectra and elemental analysis confirmed molecular structure and TLC checked the purity of the investigated derivative. Thin-layer chromatography was carried out on commercial Merck SiO\(_2\) 60 F\(_{254}\) plates having a fluorescence indicator. The spots were visualised with UV light \(\lambda = 254\) and 355.

**Material and Methods**

**Cell cultures.** Vero (GMK, Green Monkey Kidney cells) were obtained from the European Collection of Cell Cultures (ECACC 88020401). HEK-293 (human embryonic kidney cells) derived from the American Type Culture Collection (ATCC CRL-1573). HEK 293 cells were generated by the transformation of human embryonic kidney cell cultures with sheared adenovirus 5 DNA. They were grown in Eagle’s Minimal Essential Medium (MEM, Sigma) supplemented with 10% foetal bovine serum (FBS, Sigma) and 100 U mL\(^{-1}\) of penicillin (Polfa, Tarchomin) and 100 \(\mu\)g mL\(^{-1}\) of streptomycin (Polfa, Tarchomin). The cell culture was incubated in a humidified atmosphere with 5% of CO\(_2\). The investigations were carried out in the Department of Virology, Medical University, Lublin.

**Toxicity in cell cultures.** The investigated compound was dissolved in dimethylsulfoxide (DMSO, Sigma) 10 \(\mu\)g mL\(^{-1}\) and then diluted in cell culture media supplemented with 2% foetal bovine serum (FBS). GMK and HEK cells were plated into 96-well plastic plates (Nunc, Denmark) at a cell density 2 x 10\(^4\) cells per well in Eagle’s Minimal Essential Medium (MEM, Sigma) supplemented with 10% foetal bovine serum (FBS, Sigma) and 100 U mL\(^{-1}\) of penicillin (Polfa, Tarchomin) and 100 \(\mu\)g mL\(^{-1}\) of streptomycin (Polfa, Tarchomin). The cell culture was incubated in a humidified atmosphere with 5% of CO\(_2\). The investigations were carried out in the Department of Virology, Medical University, Lublin.

**Results and Discussion**

The toxicity of the investigated compound towards GMK cells was dependent on its dose and time of incubation (Table 1).

Furthermore, the toxicity of the tested derivative depended significantly on the presence of serum. When medium supplemented with 2% foetal bovine serum (FBS) was used, the cytotoxicity of the tested heterocycle was several-fold lower than in medium without FBS (data not shown).

<table>
<thead>
<tr>
<th>Concentration ((\mu)g mL(^{-1}))</th>
<th>Viability of cells (%)</th>
<th>Ad-5 titre (TCID(_{50}) mL(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GMK</td>
<td>HEK-293 (18)</td>
</tr>
<tr>
<td>24 h</td>
<td>48 h</td>
<td>72 h</td>
</tr>
<tr>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.5</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>25</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>50</td>
<td>100</td>
<td>90</td>
</tr>
<tr>
<td>100</td>
<td>90</td>
<td>80</td>
</tr>
<tr>
<td>200</td>
<td>10</td>
<td>50</td>
</tr>
</tbody>
</table>

The titre of the virus was found to be 2x10\(^4\) TCID\(_{50}\) mL\(^{-1}\). The suspension of the virus was diluted in media without foetal bovine serum (FBS, Sigma) to final concentration of 50, 100 and 200 \(\mu\)g mL\(^{-1}\). Mixtures were incubated at 37°C for 1 h. Then the virus was titrated in the appropriate cell culture (Ad-5 in a hypotriploid human cell line - HEK-293). The virus suspension with media but without tested derivative was a control. The cytopathic effect of the virus (CPE) occurring after 24 h of incubation was determined by using the Reed-Muench method (11). Antiviral action of the tested derivative was performed by the cytopathic effect (CPE) inhibition assay (12). The presented results were obtained from three independent measurements. The investigations were carried out in the Department of Virology, Medical University, Lublin.
The investigated compound in concentration ranges from 2.5 to 25 µg mL\(^{-1}\) was totally non-toxic for GMK cells after exposure for 24-120 h. Moreover, the tested compound at the concentration of 50 µg mL\(^{-1}\) and 100 µg mL\(^{-1}\) was found to be non-toxic and almost non-toxic for GMK cells after exposure for 24-48 h. However, in the highest tested concentration (200 µg mL\(^{-1}\)) this heterocycle caused significant viability decreases in GMK cells.

On the contrary, in our previous studies (18) the investigated derivative possessed virucidal activity against Ad-5, belonging to DNA viruses from the Adenoviridae family. During 1 h of contact with the virus, this derivative caused the decrease in its titre by 0.56 log, which correspond to the level of inhibition – 17.3%. The investigated compound in concentration ranges from 2.5 to 25 µg mL\(^{-1}\) was found to be completely non-toxic for normal human embryonic kidney (HEK-293) cells after exposure for 24-120 h. Furthermore, this agent at concentrations of 50 and 100 µg mL\(^{-1}\) was completely non-toxic for HEK-293 cell line after exposure for 24-48 h. Similarly, in the highest tested concentration (200 µg mL\(^{-1}\)) this heterocycle evoked only slightly viability decreases (to 90-95%) in HEK-293 cells after incubation for 24-120 h (Table 1). So, it has been approved as a good lead compound that warrants further investigation.

The conducted experiments revealed that the examined derivative possessed virucidal activity against Ad-5, belonging to DNA viruses from the Adenoviridae family. During 1 h of contact with the virus, this compound caused the decrease in its titre by 0.56 log, which correspond to the level of inhibition – 17.3% (Table 1), independently from the applied concentration (50, 100 and 200 µg mL\(^{-1}\)).

In conclusion, 6-(2,3-dimethylphenyl)-7,8-dihydro-6H-imidazo[1,2-d][1,2,4]triazolo[4,3-b] [1,2,4]triazole-3(2H)thione, was found to be non-toxic for human embryonic kidney cells and demonstrated inhibitory properties against Ad-5, justifying its further investigation as possible non-toxic antiviral agent.

References

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