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BIOCHEMISTRY, BIOPHYSICS, AND MOLECULAR BIOLOGY

Fluorinated Derivatives of Benz[4,5]imidazo[1,2-*b*][1,3] thiazole—Inhibitors of Reproduction of Measles Virus

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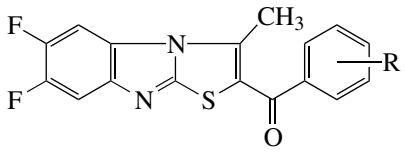
Currently, designing antiviral drugs remains a topical problem because of a wide distribution of HIV infection, viral hepatites, and appearance of new viral infections (including those induced by coronaviruses). The special attention of researchers is attracted by the fluorine-containing heterocyclic compounds, which exhibit a unique set of proportions, because the presence of the fluorine atom increases their solubility in lipids and ability to penetrate across cell membranes [1]. The compounds of the benzimidazole series occupy a key position among the known drugs. For example, the spasmolytic dibazol, neuroleptics pimozide and droperidol, antihistamine drug astemizol, and highly effective antiulcerogenic drug omeprazol are widely used in medicine [2]. Benzimidazole derivatives (including fluorine-containing compounds) that exhibit antiviral activity against herpes virus were found [3–5].

In this work, we studied the antiviral activity of new fluorine-containing derivatives of benz[4,5]imidazo[1,2-*b*][1,3]thiazoles (**1a–1n**) against the measles virus. The synthesis of the compounds analyzed was described by us earlier [6].



R = H (**1a**); $NHCOCH_3$ (**1b**);, 4-Cl (**1c**); 4-Br (**1d**); 4-OH (**1e**); 4-$OCOCH_3$ (**1f**); 4-NO_2 (**1g**); 3-NO_2 (**1h**); 2-CH_3 (**1i**); 2,4-Cl_2 (**1j**); 3,4-Cl_2 (**1k**); 2,4-$(OCH_3)_2$ (**1l**); 3,4-$(OCH_3)_2$ (**1m**); 4-Ph (**1n**); X = Cl, Br.

Antiviral activity of these compounds was studied *in vitro* in Vero green marmoset kidney cells against the measles virus strain Leningrad 16. Cells were cultured in 96-well plates (Costar, the United States) in a DMEM nutrient medium supplemented with 2% bovine fetal serum (ICN, the United States), 100 μg/ml gentamycin, and 60 μg/ml lincomycin at 37°C in 5% CO_2. When monolayer was formed, the virus (infection multiplicity, 0.2–0.5 infective units per cell) and the compound tested (concentration, 0.1–400 μg/ml) were added to wells. Cells were incubated at 37°C for 5 days in 5% CO_2 and then stained with crystalline violet (1.3 g of the dye, 50 ml of ethanol, water to 700 ml, and 300 ml of 40% formalin) for 1.5 h. Optical density was determined on a spectrophotometer at a wavelength of 570 nm. Based of the data obtained, 50% toxic (CD_{50}) and effective (ED_{50}) doses of compounds were calculated [7]; the therapeutic index (IS) was determined as CD_{50}/ED_{50}.

The results of study of antiviral activity and cytotoxicity of 2-aroyl-3-methyl-6,7-difluorobenz[4,5]imidazo[2,1-*b*][1,3]thiazoles (**1a–1n**) are summarized in Table 1. Ribavirin (1-(β-d-ribofuranosyl-10-1,2,4-triazol-3-carboxamide)), which is commonly used for treating infections induced by RNA-containing viruses (including heavy forms of measles) [8], was used as a reference drug. However, the use of this drug is limited by its high toxicity. Out of 14 compounds studied, compound **1a** exhibited the greatest activity: its therapeutic index was much greater than that of ribavirin (245.5 and 14.4, respectively). Lower activity was exhibited by compound **1b**; the other compounds had low activity or were ineffective.

We studied the antiviral activity of compound **1a** depending on the time of its addition to culture medium. The tested compound was added one day before virus adsorption, simultaneously with the virus, immediately after virus adsorption, and 6 h and one day after virus adsorption. The results of this experiment are shown in Table 2. It is seen that preliminary treatment of cells with the compound did not protect cells

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Table 1. Antiviral activity and cytotoxicity of 2-aroyl-3-methyl-6,7-difluorobenz[4,5]imidazo[2,1-*b*][1,3]thiazoles (**1a–1n**)

Compound	R	CD_{50}, μg/ml	ED_{50}, μg/ml	IS
Ribavirin		180	12.5	**14.4**
1a	H	81	0.33	**245.5**
1b	$NHCOCH_3$	50	6.25	8
1c	4-Cl	80	n/inactive	
1d	4-Br	>100	100	>1
1e	4-OH	>100	100	>1
1f	$4\text{-}OCOCH_3$	>100	n/inactive	
1g	$4\text{-}NO_2$	50	n/inactive	
1h	$3\text{-}NO_2$	20	n/inactive	
1i	$2\text{-}CH_3$	>200	n/inactive	
1j	$2,4\text{-}Cl_2$	>100	n/inactive	
1k	$3,4\text{-}Cl_2$	20	n/inactive	
1l	$2,4\text{-}(OCH_3)_2$	>100	90	>1.1
1m	$3,4\text{-}(OCH_3)_2$	10	n/inactive	
1n	4-Ph	>100	90	>1.1

Table 2. Activity of compound **1a** against measles virus depending on the time of its addition to culture medium

Time of addition of compound **1a**	CD_{50}, μg/ml	ED_{50}, μg/ml	IS
One day before infecting with virus	81	>81	<1
Simultaneously with virus	81	0.33	245.5
Immediately after virus adsorption	81	0.7	115.7
Six hours after infecting with virus	81	1	81
One day after infecting with virus	81	1.1	73.5

against subsequent infection with the measles virus. However, addition of the tested compound simultaneously with the virus or after infecting only slightly affected its antiviral activity: ED_{50} in the case of simultaneous addition was only in 3, 4 times greater than ED_{50} determined when the drug was added one day after infecting.

Thus, the study of antiviral activity of 2-aroyl-3-methyl-6,7-difluorobenz[4,5]imidazo[2,1-*b*][1,3]thiazoles (**1a–1n**) *in vitro* showed that 2-phenyl-3-methyl-6,7-difluorobenz[4,5]imidazo[2,1-*b*][1,3]thiazole (**1a**) exhibits a pronounced activity against the measles virus and a low toxicity for Vero cells. The antiviral activity slightly changed depending on the time of its addition to culture medium, when the compound was added simultaneously with the virus or within one day after infecting. Apparently, the antiviral activity of compound **1a** is related to blocking late stages of measles virus reproduction (synthesis or assembling of viral proteins). In view of this, the drug based on this compound may be used both as a therapeutic and prophylactic agent.

Thus, further study of compound **1a** and the whole group of fluorine-containing benzimidazole derivatives show promise for designing novel drugs effective against viral infections.

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