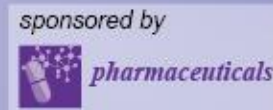




# 4th International Electronic Conference on Medicinal Chemistry

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chaired by Dr. Jean Jacques Vanden Eynde



## **Antiviral effect of derivatives of triazoles on EBV-associated lymphoblastoid cells**

Krystyna Naumenko<sup>1\*</sup>, Anna Golovan<sup>1</sup>, Galina Baranova<sup>1</sup>, Svitlana Zagorodnya<sup>1</sup>,  
Anna Gudz<sup>2</sup>, Yurii Shermolovych<sup>2</sup>

<sup>1</sup>Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, Acad. Zabolotny str., 154, Kyiv, 03143, Ukraine

<sup>2</sup>Institute of organic chemistry, National Academy of Sciences of Ukraine, Murmanska 5 Str., Kyiv, Ukraine, 02660

\* Corresponding author: [krystyn.naumenko@gmail.com](mailto:krystyn.naumenko@gmail.com)

## Abstract:

Epstein–Barr virus (EBV) causes lymphocyte-proliferative diseases, such as Burkitt's lymphoma, Hodgkin's lymphoma, other B and T cell lymphomas. Recently the connection between EBV and autoimmunity diseases has been demonstrated. In recent years, several studies have explored the concept that the compounds that have anti-herpetic activity might be able to influence the cell cycle of infected cells, by eliminating them from the body. However, cell cycle regulation during EBV-infection and the effect of anti-EBV drugs have received only limited attention.

The aim of our work was to study derivatives of triazole (**G14**, **G20**, **G22**, and **G24**) as potential antiherpetic agents and their effect on the cell cycle of lymphoblastoid cell lines B95-8. According to PCR, anti-EBV activity was observed only for compounds **G14** and **G22**, EC<sub>50</sub> values were 27 and 100 µg/ml. The B95-8 cells treated with all studied compounds were analyzed with the help of flow cytometry (cells were stained with propidium iodide). It was observed an induction of apoptosis in the presence of **G22** at 700 µg/ml; the proportion of apoptotic cells reached almost 40%. Other compounds **G14** and **G24** led to the switch of cells from the Sub G0 phase of the cell cycle to the G1 phase and subsequent activation of the S-phase. These compounds may play an important role as potential inducers of EBV lytic infection; with the addition of antiherpesvirus drugs, they could be therapeutically beneficial for EBV-associated tumors.



# Introduction

The Epstein–Barr virus (EBV) was discovered 54 years ago by electron microscopy of cells cultured from Burkitt's lymphoma tissue. EBV is present worldwide and infects more than 90% of the human population. EBV, also known as human herpesvirus 4, is a gamma-herpes virus that is usually acquired silently early in life and carried thereafter as an asymptomatic infection of the B lymphoid system. Also, EBV is associated with nasopharyngeal carcinoma, Hodgkin's lymphoma, post-transplant lymphoma and nasal-type NK/T-cell lymphoma as well as other diseases. Recently it was shown that there is a link between EBV and autoimmunity disorders, such as systemic lupus erythematosus.

Latent and chronic EBV infection allows the long-term persistence of infected cells that can avoid the host antiviral immune response and block apoptotic death of infected cells. Thus, the proliferation of latently infected EBV cells would lead to an increase of the infected B-cell population.

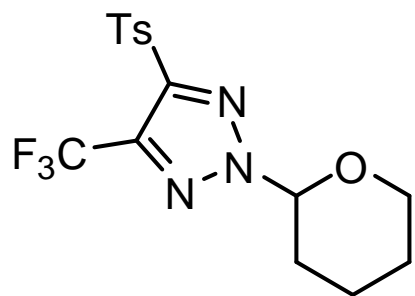
Over decades highly active fluorinated nucleosides have been synthesized and used in cancer and viral treatment. The study of fluorinated analogs of nucleosides has led to the development of novel promising chemotherapeutic agents.

Thus, there is an active search for compounds that have anti-EBV activity and may induce apoptosis of cells. Also, compounds that can influence the cell cycle of infected cells are of interest.



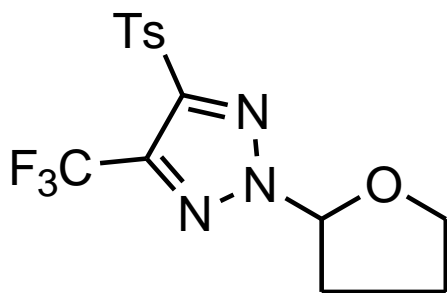
# Fluorinated derivatives of triazole

2-(tetrahydro-2*H*-  
pyran-2-yl)-4-tosyl-  
5-(trifluoromethyl)-  
2*H*-1,2,3-triazole  
(G14)



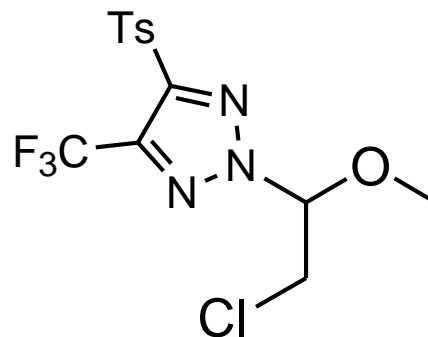
M.m. 373,35

2-(tetrahydrofuran-2-  
yl)-4-tosyl-5-  
(trifluoromethyl)-2*H*-  
1,2,3-triazole  
(G20)



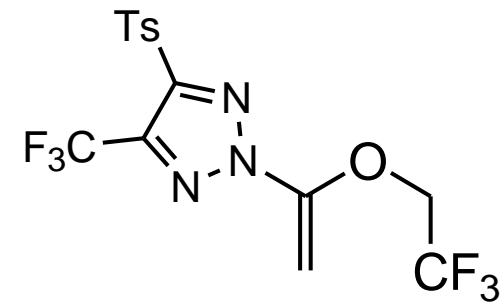
M.m. 361.34

2-(2-chloro-1-  
ethoxyethyl)-4-tosyl-  
5-(trifluoromethyl)-  
2*H*-1,2,3-triazole  
(G22)



M.m. 397.8

4-tosyl-2-(1-(2,2,2-  
trifluoroethoxy)vinyl)-  
5-(trifluoromethyl)-  
2*H*-1,2,3-triazole  
(G24)



M.m. 415.31

All studied compounds were synthesized in Institute of Organic Chemistry of NAS of Ukraine



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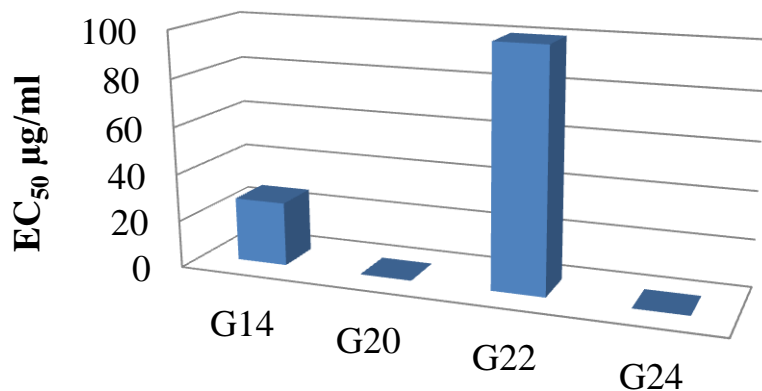


pharmaceuticals

# Results and discussion

Study of the cytotoxicity and antiviral action of the abnormal nucleoside analog **G14**, **G20**, **G22**, and **G24** were performed with Raji cell line (EBV positive human B-cells) superinfected by EBV, as an acute infection model.

The cytotoxicity of compounds was determined by MTT-method,  $CC_{50}$  were in the range 300 -1000  $\mu\text{g/ml}$ . The antiviral activity of the compounds was determined by RT-PCR. It was shown that only **G14** and **G22** compounds were effective to inhibit EBV reproduction; the  $EC_{50}$  values were 27  $\mu\text{g/ml}$  and 100  $\mu\text{g/ml}$ . The selective indices (SI) for these compounds were in the range 3 - 17.



	G14	G20	G22	G24
■ $EC_{50}$	27	0	100	0

Compound	$CC_{50}$	$EC_{50}$	IS
<b>G14</b>	465	27	17
<b>G20</b>	400	n/a	-
<b>G22</b>	300	100	3
<b>G24</b>	1000	n/a	-

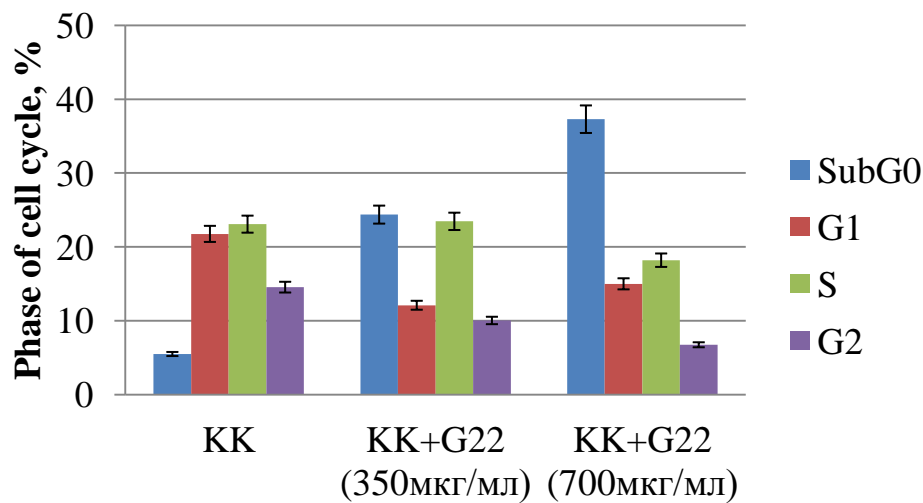
n/a – not active



EBV leads to the transformation of B-cells, which provides lifetime persistence of virus-infected cells in the host's organism. It is one of the mechanisms of development of lymphoproliferative diseases. One of the aspects of modern anticancer therapy is the search for apoptosis-stimulating compounds. For this purpose B95-8 (EBV-producing B-cell line) was used as a chronic infection model. The cell fluorescence intensity was measured by flow cytometry, samples were stained by the solution of PBS that contained RNase (100  $\mu\text{g/ml}$ ) and propidium iodide (PI, 50  $\mu\text{g/ml}$ ).

It was shown that compound **G22** at concentration 700  $\mu\text{g/ml}$  induced an increase of the percentage of the apoptotic cells on 48 hours incubation; the proportion of apoptotic cells reached almost 40%. The minimum concentration of the studied compound (350  $\mu\text{g/ml}$ ) leads to an increase in the percentage of apoptotic cells.

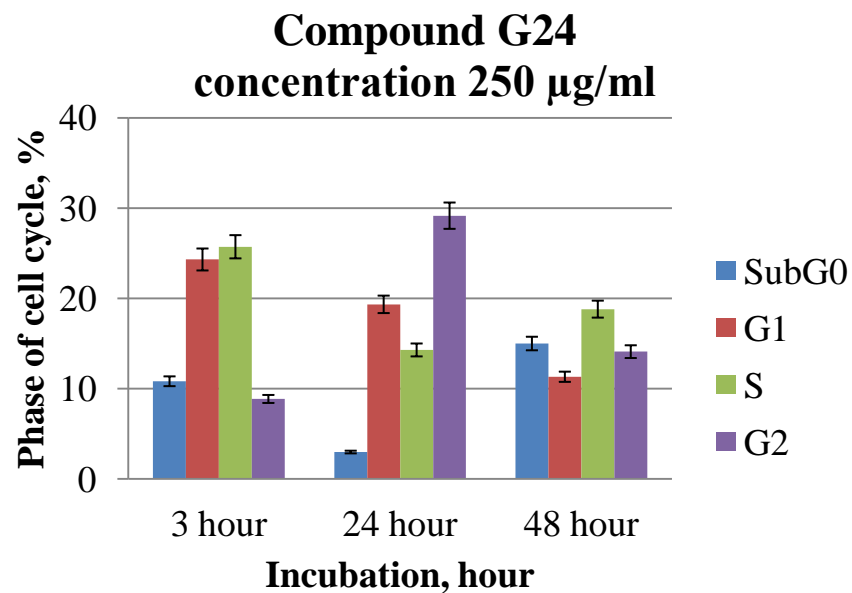
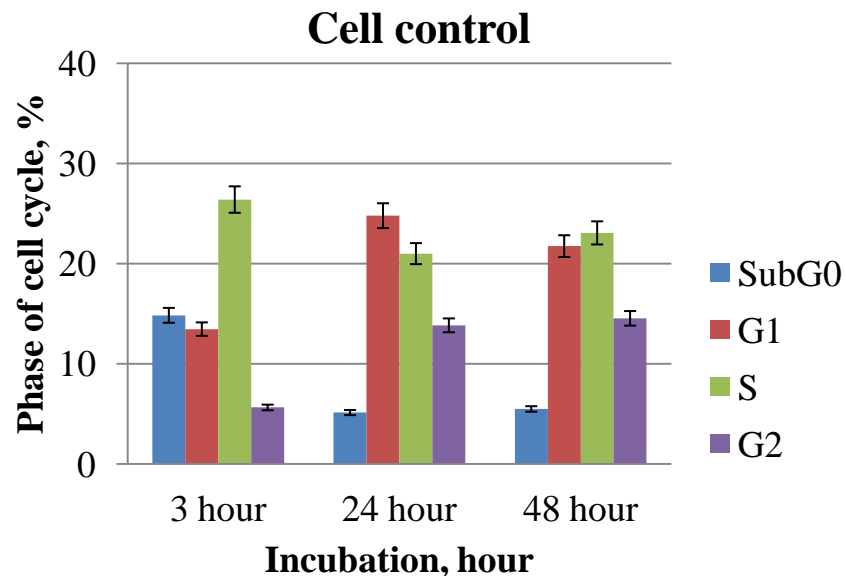
#### 48 hours



During a chronic EBV-infection, an infectious virus is synthesized in 20% of the cell population. It complicates the treatment of this form of infection. One of the way to treat EBV infected cells is to induce lytic infection with addition anti-EBV drugs.

It was established that compound **G24** do not influence the cell cycle after 3-hour incubation. After 24 hours, number of cells in the G1 and S phases decreased (19% and 17%), but the percentage of cell in the G2 phase increased to 29%. It was shown that the number of cells in the subG0 phases of the cell cycle increased (15%) after 48h. Also inhibition of another phase of cell cycle was detected.

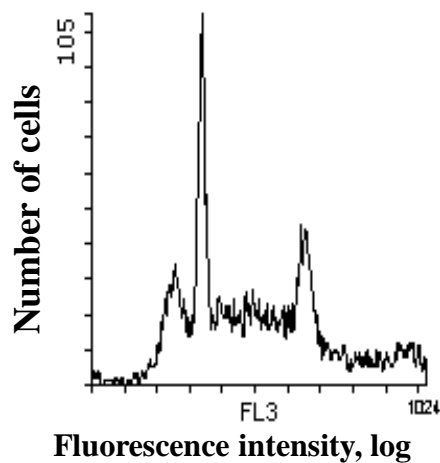
Results showed that compound **G24** might induce changes from G1 phase to S phase and next replication of the cell. It is may be used for inducing lytic infection and treatment of such cells with antiviral drugs.



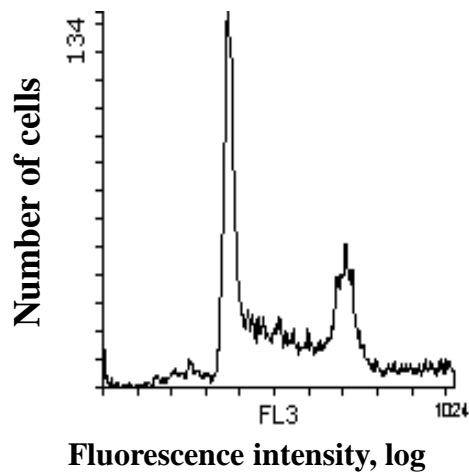
Here presented histograms cell control and cell with addition of compound G24.

### Time of incubation, hour

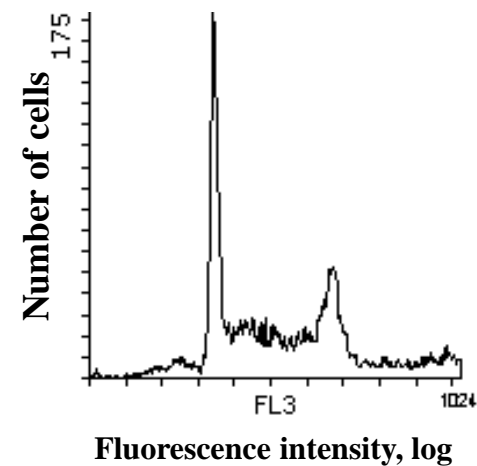
3



24

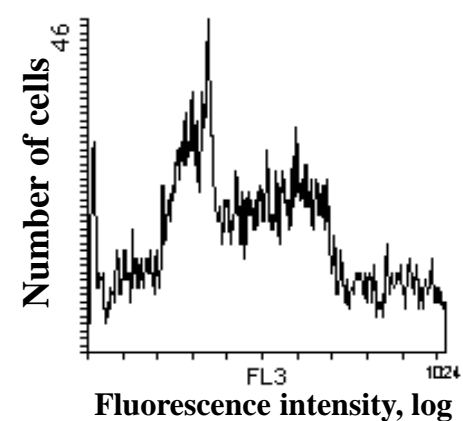
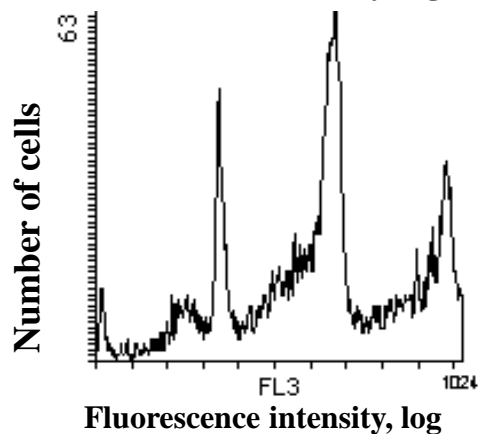
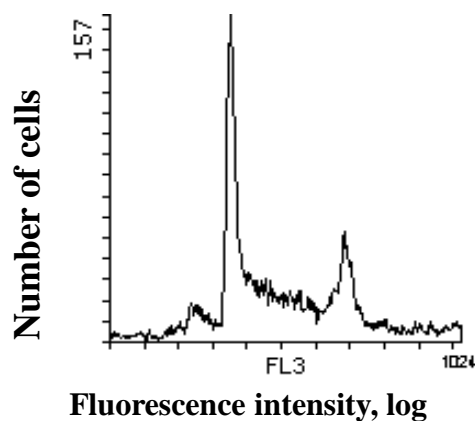


48



Cell control

Compound G24  
(500 µg/ml)





## Conclusions

Analysis of the antiviral activity of the compounds **G14** and **G22** demonstrated a high level of activity against EBV,  $EC_{50}$  were 27 and 100  $\mu\text{g}/\text{ml}$ . Also, we detected the significant impact on the growth of transformed cells with the addition of compound **G22**. It can be assumed that the destruction of cells infected with virus occurs through apoptosis.

Another way to treat EBV infected cells is to induce lytic infection with addition anti-EBV drugs. Thus, compound **G24** influences the cell cycle of B95-8 cell line, resulting into the switch of cells from the SubG0 phase of the cell cycle to the G1 phase and subsequent activation of the S-phase. This may lead to the induction of a lytic infection, in which a large number of viral proteins are synthesized.

Obtained data allow considering these compounds as perspective antiviral and antitumor agents.

