Available online at www.sciencedirect.com

ScienceDirect

Mendeleev Commun., 2021, **31**, 667–669

Mendeleev Communications

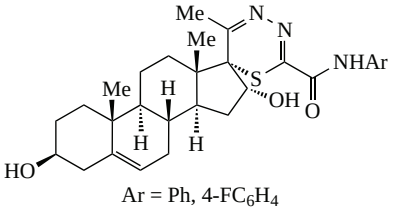
New synthetic corticosteroids inhibit Epstein–Barr virus release

Andrei G. Malykh,[a] Andrey R. Pavlov,[a] Alexander V. Komkov,[b] Yulia A. Volkova,[b] Leonid G. Menchikov[b] and Igor V. Zavarzin*[b]

[a] *Capital Biosciences, Inc., MD 20878, USA*
[b] *N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 119991 Moscow, Russian Federation. E-mail: zavi@ioc.ac.ru*

DOI: 10.1016/j.mencom.2021.09.025

Novel spiroandrostene-17,6′-[1,3,4]thiadiazines obtained by synthesis from 16β,17β-epoxy-17-isopregn-5-en-3β-ol-20-one and *N*-aryl-2-hydrazino-2-thioxoacetamide efficiently inhibited the release of Epstein–Barr virus from cells carrying the virus in culture. Other studied steroids, such as Spirolactone, Digitonin, Diosgenin, and Hecogenin were inactive. DMSO alone was found to inhibit the virus release, as well, likely, because of changing properties of the cell membranes. The novel steroids, Spironolactone and most analogues display no or low cell toxicity while Digitonin produced a significant toxic effect.



Ar = Ph, 4-FC_6H_4

Keywords: steroids, androstene, 1,3,4-thiadiazine, spironolactone, Epstein–Barr virus, antiviral activity.

Epstein–Barr virus (EBV), a major human *Herpesviridae*, was discovered over 50 years ago as the first human tumour virus.[1,2] Since finding it in Burkitt lymphoma, EBV was associated with other malignancies, infectious mononucleosis and autoimmune illnesses. EBV contributes to about 1.5% of all cases of human cancer worldwide and 1.8% of all cancer deaths.[1–4] So far, no effective vaccine against EBV is available, and its treatment is limited by the use of compounds such as acyclovir.

Recently, it was reported that Spironolactone (SPR), known as steroid diuretic drug used to block mineralocorticoid activity, also reduced the production of EBV by targeting the SM protein.[5] This effect of SPR was found to be independent of its antimineralocorticoid effect. Later, the inhibitory activity of SPR against other type of *Herpesviridae*, equid alpha-herpesviruses (EHV), was demonstrated, as well.[6,7] Also, the antiviral effect of Spironolactone was reported for other RNA viruses HIV-1 and HIV-2.[8] Recently, Spironolactone activity was found against SARS-CoV-2 and SPR was suggested for COVID-19 therapy.[9–14] A long history of more than half a century of daily use of SPR as a diuretic and antihypertensive, and also as an inhibitory androgen drug, confirms that it is not toxic for human cells and it does not affect normal cells in long-term treatments. These observations highlight the potential advantage of using SPR and its analogues for the treatment of viral infections,[8] including EBV and HIV.

Spironolactone is a synthetic corticosteroid and a distinctive element of its structure is a heterocycle spiro-linked to the 17-position of the D-ring of steroid core (Figure 1). In this study we assumed that such steroidal compounds bearing spiro heterocycle could be promising for development of novel

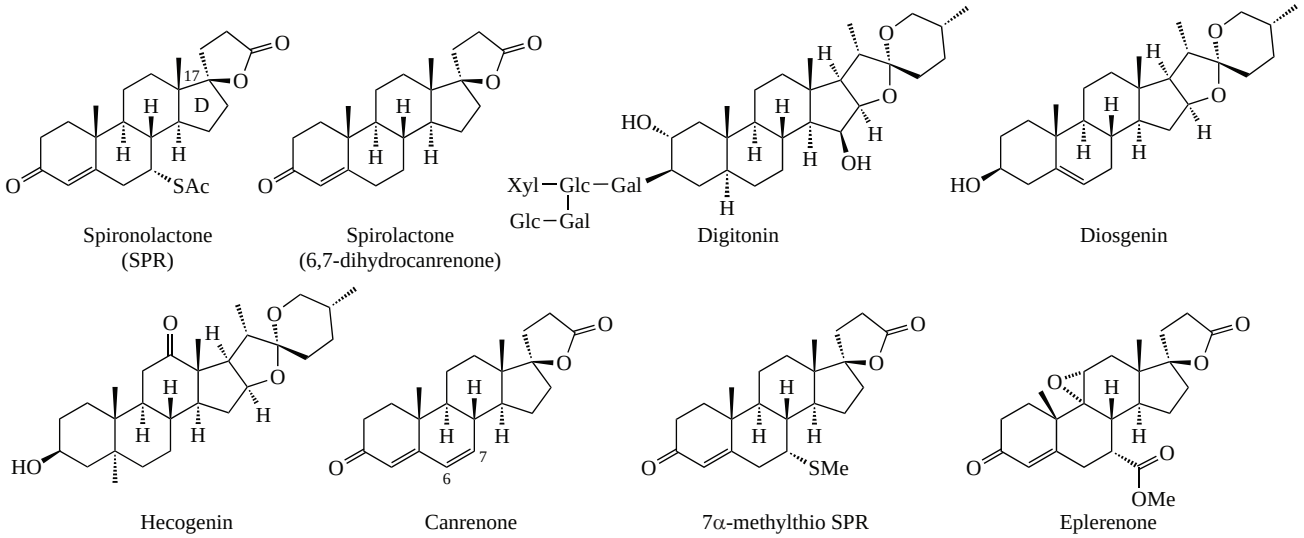
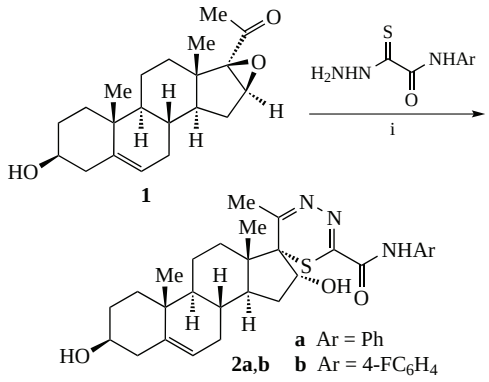


Figure 1 Spironolactone and its analogues used in this study.



Scheme 1 *Reagents and conditions*: i, TsOH, 1,4-dioxane, 40 °C, 10 h, yield 60–65%.

antiviral agents. In particular, we tested the antiviral activity of two novel spiroandrostene-17,6′-[1,3,4]thiadiazines bearing [1,3,4]thiadiazines spiro-attached at the 17-position (Scheme 1). The synthesis of new 5′-methyl-[2′-(*N*-arylcarbamoyl)]-3β,16β-dihydroxyspiroandrost-5-ene-17,6′-[1,3,4]thiadiazines **2a**,**b** was carried out according to a modified method[15] by the reaction of 16β,17β-epoxy-17-isopregn-5-en-3β-ol-20-one **1** and *N*-aryl-2-hydrazino-2-thioxoacetamides in the presence of *p*-toluenesulfonic acid at 40 °C. The reaction proceeded with the reversal of the stereochemistry at the C(16) atom and resulted in products **2a**,**b**.

Verma *et al*., along with SPR, studied the antiviral effect of two SPR metabolites, canrenone and 7α-thiomethyl SPR, as well as eplerenone (see Figure 1).[5] Canrenone exhibited antiviral activity, although lower than that of SPR, whereas 7α-thiomethyl SPR and eplerenone did not.[5] The chemical structure of canrenone and 7α-thiomethyl SPR is different by the modifications at C(7) atom of SPR. For this reason, it was suggested that the nature of a substituent at C(7) of SPR is crucial for the antiviral activity.[5] However, such a comparison of SPR with canrenone (a formal product of thioacetic acid elimination from SPR) is not completely valid, since canrenone, unlike SPR, has an additional double bond between C(6) and C(7), whereby the donor-acceptor effects of the substituents at C(3) can be transferred through a system of conjugated bonds. Therefore, along with canrenone, the antiviral activity of spirolactone (6,7-dihydrocanrenone), whose molecule does not contain this double bond, should have been assessed. In addition, saponins such as Digitonin, Diosgenin, and Hecogenin, as compounds with 1,6-dioxaspiro[4.5]decane fragment annulated to D ring of the androstane core, attracted our attention for testing their antiviral activity. Our current results show a significant potential for the use of SPR in treatment of viral infections, as well as discover new antiviral drug candidates among SPR analogue compounds.

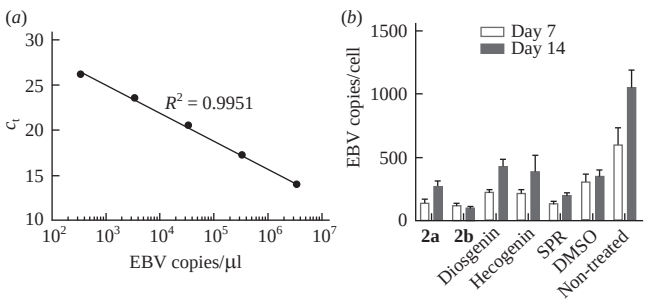


Figure 2 (*a*) Standard curve obtained with EBNA1 primers in qPCR on EBV DNA serially diluted with cell growth medium. (*b*) Interpolated virus copy numbers at days 7 and 14 were normalized per live cell. The mean ±SD of PCR duplicates for three wells is shown.

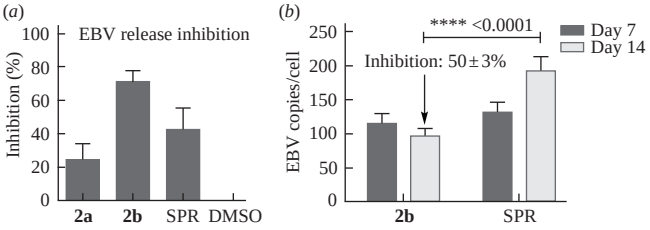


Figure 3 (*a*) Estimated inhibition rates compared to DMSO on day 14. (*b*) Comparison of EBV release after treatment with **2b** and Spironolactone. Statistical significance calculated by Student's T-test is indicated as: **** = $p < 0.0001$.

In our experiments on antiviral activity, Epstein–Barr virus producing B95-8 cells have been were treated with the substances of interest, and the release of virus particles was determined by qPCR with the primers specific to EBV DNA. All steroids were tested at a concentration of 25 μM that produced the maximal inhibitory effect for SPR. To compare effects of the tested compounds, the numbers of virus copy were evaluated from the standard curve obtained for EBV DNA diluted with the cell growth medium [Figure 2(*a*)]. EBV copy numbers were normalized by the numbers of viable cells in each well at days 4, 7, 11 and 14 of the incubation with the substances. All compounds exhibited anti-EBV activity with respect to the non-treated control [Figure 2(*b*)]. In addition, DMSO alone at concentration of 0.025% appeared to inhibit EBV release by over 50%, as compared to the non-treated cells. Also, normalized numbers for Digitonin appeared significantly higher than those for non-treated control (not shown here) suggesting increased release of the virus into the media. Therefore, we excluded these two compounds from further studies. Two candidates **2a** and **2b** demonstrated inhibitory activity as compared to DMSO treated control [Figure 3(*a*)]. The corticosteroid **2b** appeared to be more effective than SPR, with ~50% higher inhibition rate at day 14 of treatment [Figure 3(*b*)].

Our test showed that Spirolactone, in contrast to canrenone, was not active. Thus, it can be argued that in SPR derivatives the absence of substituents at C(7) as well as the double bond between C(6) and C(7) are crucial for antiviral activity. We also examined known steroidal saponins structurally related to Spironolactone, such as Digitonin, Diosgenin, and Hecogenin (compounds with 1,6-dioxaspiro[4.5]decane fragment annulated to D-ring of androstane core), and found them inactive against EBV. All studied compounds were tested for cell toxicity (for details, see Online Supplementary Materials). We found that the cell growth rate were similar for most compounds tested. Specifically, the novel steroidal thiadiazines **2a**,**b**, SPR and most their analogues showed no or low cell toxicity. In contrast, Digitonin exhibited significant effect on cell viability and caused high cell death rate at 25 μM by day 14. Our discovery of the EBV-inhibitory activity of novel steroidal thiadiazines **2a**,**b** opens the way for the development of novel anti-EBV strategies without the limitations of anti-viral therapy of drug resistance and high cost. These compounds, by analogy with SPR, can also be considered as candidates for studying their antiviral activity against HIV and SARS-CoV-2.

In summary, we have shown that the obtained steroid 1,3,4-thiadiazines inhibit EBV release. The fluorine-substituted corticosteroid **2b** appeared the most promising anti-EBV agent, as compared to Spironolactone. Steroidal saponin Digitonin appeared toxic to B95-8 cells at 25 μM concentration. Finally, we found that DMSO alone inhibit the EBV release. This study opens new directions for search and development of anti-EBV drugs.

The authors are grateful to R. A. Novikov (N. D. Zelinsky Institute of Organic Chemistry) for his assistance in carrying out NMR analyses.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2021.09.025.

References

1 *Epstein Barr Virus Volume 1: One Herpes Virus: Many Diseases*, ed. C. Münz, Springer International Publishing, Switzerland, 2015.
2 *Epstein Barr Virus Volume 2: One Herpes Virus: Many Diseases*, ed. C. Münz, Springer International Publishing, Switzerland, 2015.
3 P. J. Farrell, *Annu. Rev. Pathol.: Mech. Dis.*, 2019, **14**, 29.
4 L. S. Young, L. F. Yap and P. G. Murray, *Nat. Rev. Cancer*, 2016, **16**, 789.
5 D. Verma, J. Thompson and S. Swaminathan, *Proc. Natl. Acad. Sci. USA*, 2016, **113**, 3609.
6 C. J. Thieulent, E. S. Hue, C. I. Fortier, P. Dallemagne, S. Zientara, H. Munier-Lehmann, A. Hans, G. D. Fortier, P.-H. Pitel, P.-O. Vidalain and S. L. Pronost, *Virology*, 2019, **526**, 105.
7 C. Thieulent, E. S. Hue, G. Sutton, C. Fortier, P. Dallemagne, S. Zientara, H. Munier-Lehmann, A. Hans, R. Paillot, P.-O. Vidalain and S. Pronost, *Antiviral Res.*, 2020, **183**, 104931.
8 B. Lacombe, M. Morel, F. Margottin-Goguet and B. C. Ramirez, *J. Virol.*, 2016, **90**, 10972.
9 F. A. Cadegiani, *Am. J. Physiol.: Endocrinol. Metab.*, 2020, **318**, E587.
10 F. A. Cadegiani, *BMC Endocrine Disorders*, 2020, **20**, 149.
11 F. A. Cadegiani, A. Goren and C. G. Wambier, *Med. Hypotheses*, 2020, **143**, 110112.
12 L. Lisi, P. M. Lacal, M. L. Barbaccia and G. Graziani, *Biochem. Pharmacol.*, 2020, **180**, 114169.
13 L. Liaudet and C. Szabo, *Crit. Care*, 2020, **24**, 318.
14 O. Altay, E. Mohammadi, S. Lam, H. Turkez, J. Boren, J. Nielsen, M. Uhlen and A. Mardinoglu, *iScience*, 2020, **23**, 101303.
15 A. S. Komendantova, A. M. Scherbakov, A. V. Komkov, V. V. Chertkova, A. O. Gudovanniy, E. I. Chernoburova, D. V. Sorokin, Y. U. Dzichenka, V. Z. Shirinian, Y. A. Volkova and I. V. Zavarzin, *Bioorg. Chem.*, 2019, **91**, 103142.

Received: 22nd April 2021; Com. 21/6537