ISSN 1561-8323 (Print)
ISSN 2524-2431 (Online)

UDC 547.92:615.281.8
https://doi.org/10.29235/1561-8323-2023-67-4-295-299

Received 02.12.2022
Поступило в редакцию 02.12.2022

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ANTI-CORONAVIRUS PROPERTIES OF BRASSINOSTEROIDS

Abstract. Antiviral properties of natural brassinosteroids of the campestane, ergostane, and stigmastane series (6-ketones and B-lactones) and their (22*S*,23*S*)-analogs were studied using the seasonal human respiratory alpha-coronavirus 229E (HCoV-229E) as an example. The presence of anticoronavirus properties was shown for a number of studied compounds. In general, 6-ketones were more active than B-lactones. The maximum inhibitory effect (EC_{50} 21.1 μM) in relation to the reproduction of HCoV-229E was noted for (22*S*,23*S*)-epicastasterone.

Keywords: brassinosteroids, brassinolide, castasterone, coronavirus, antiviral properties, HCoV-229E

For citation. Zhabinskii V. N., Matorin A. M., Savinova O. V., Boreko E. I., Khripach V. A. Anti-coronavirus properties of brassinosteroids. *Doklady Natsional'noi akademii nauk Belarusi = Doklady of the National Academy of Sciences of Belarus*, 2023, vol. 67, no. 4, pp. 295–299. https://doi.org/10.29235/1561-8323-2023-67-4-295-299

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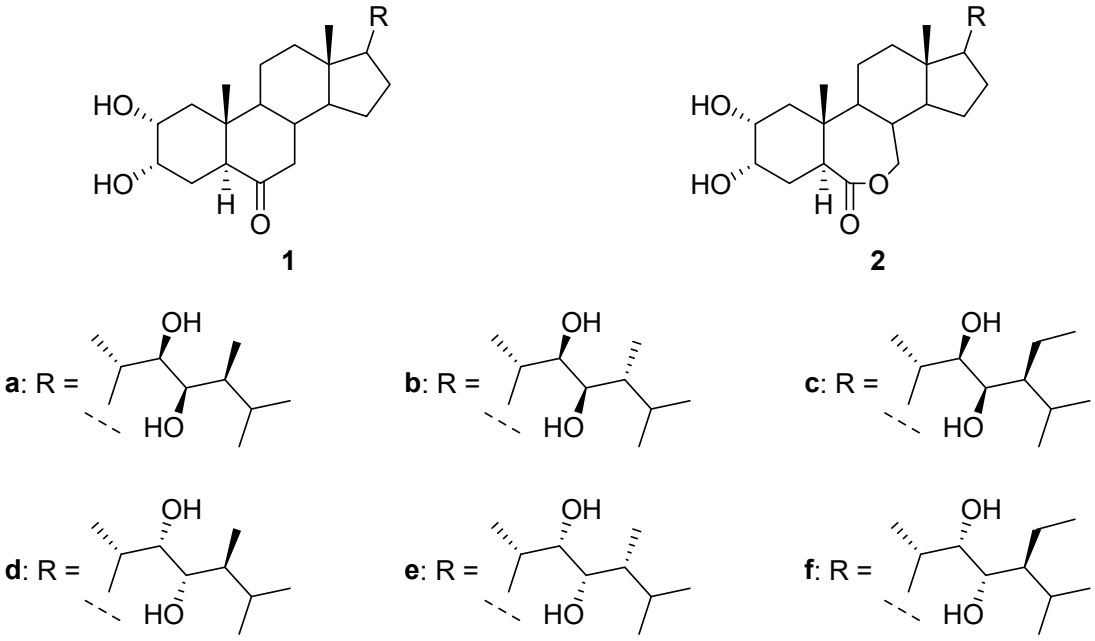
ПРОТИВОКОРОНАВИРУСНЫЕ СВОЙСТВА БРАССИНОСТЕРОИДОВ

Аннотация. На примере сезонного респираторного альфа-коронавируса человека 229E (HCoV-229E) проведено изучение противовирусных свойств природных брассиностероидов кампестанового, эргостанового и стигмастанового рядов (6-кетонов и B-лактонов) и их соответствующих (22*S*,23*S*)-аналогов. Показано наличие противокоронавирусных свойств для ряда изученных соединений. 6-Кетоны в целом оказались более активными в сравнении с B-лактонами. Максимальный показатель ингибирующего действия (EC_{50} 21,1 μM) в отношении репродукции HCoV-229E отмечен для (22*S*,23*S*)-эпикастастерона.

Ключевые слова: брассиностероиды, брассинолид, кастастерон, коронавирус, противовирусные свойства, HCoV-229E

Для цитирования. Противокоронавирусные свойства брассиностероидов / В. Н. Жабинский [и др.] // Докл. Нац. акад. наук Беларуси. – 2023. – Т. 67, № 4. – С. 295–299. https://doi.org/10.29235/1561-8323-2023-67-4-295-299

Introduction. Brassinosteroids (BS) have been known for more than 40 years as a group of phytohormones that have a deep and versatile, in particular adaptogenic and stress-protective, effect on plants [1]. A characteristic feature of BS is their ability to neutralize the negative impact of biotic and abiotic environmental factors. Viral diseases of plants are among the biotic factors. Already in the late 1990s, the ability of BS to increase the resistance of potato plants to late blight and barley to leaf diseases was shown [2], and later, antiviral effects of BS were found for a wide range of viral diseases of tobacco and rice [3]. These data prompted researchers to look for similar properties outside the plant kingdom [4]. A great deal of work in this area was conducted by Argentine scientists, who obtained interesting results in the course of studying the antiviral effect of BS against a number of animal viruses, including poliovirus, herpes simplex viruses HSV-1 and HSV-2, measles virus, vesicular stomatitis virus and the arenaviruses [5]. In order to search for a possible mechanism of the antiviral action of BS, the effect of a non-natural analog on the synthesis of viral protein in Vero cells infected with HSV-1 was studied [6]. It was shown that there were no effects in the early stages of the virus reproduction cycle and a strong inhibition in the presence of the test substance at the late stage of protein synthesis. In experiments on a mouse herpetic stromal keratitis, the antiviral effect of BS was due to their role in immune-mediated stromal inflammation [7].



Structures of brassinosteroids used for biological studies: **1a** – castasterone, **1b** – epicastasterone, **1c** – homocastasterone, **1e** – (22*S*,23*S*)-epicastasterone, **1f** – (22*S*,23*S*)-homocastasterone, **2b** – epibrassinolide, **2c** – homobrassinolide, **2e** – (22*S*,23*S*)-epibrassinolide, **2f** – (22*S*,23*S*)-homobrassinolide

Recently, we have conducted research on the preparation and study of the antiviral properties of compositions based on the natural brassinosteroids, epibrassinolide, in order to prevent and treat viral diseases in poultry. Testing of the dextrin-epibrassinolide complex on chicken embryos *in vitro* showed that this complex is an inhibitor of the avian infectious laryngotracheitis virus [8]. A pronounced protective effect of BS against human immunodeficiency virus infection was found [4]. Treatment of cells with epibrassinolide *in vitro* significantly increased their lifespan. The number of living cells in the infected culture treated with this BS was more than 50 % higher compared to the untreated control 4–5 days after infection. Moreover, on the 3rd day after infection, a significantly reduced production of virus-specific antigens on the cell surface was observed.

The Covid-19 pandemic has become an impetus for research into the antiviral activity of compounds of various classes. Therefore, it was natural to test the anti-coronavirus properties of brassinosteroids. Nine compounds were selected for biological studies (Figure). They differed in the structure of cycle B (ketones or lactones), configuration of the diol group in the side chain and an alkyl substituent at C-24 (α-Me, β-Me or α-Et). Castasterone **1a**, epicastasterone **1b**, homocastasterone **1c**, epibrassinolide **2b**, and homobrassinolide **2c** are natural brassinosteroids and other 4 compounds are their corresponding non-natural (22*S*,23*S*)-analogues.

Experimental. The test substances (synthesized in the Laboratory of Steroid Chemistry of the Institute of Bioorganic Chemistry of the National Academy of Sciences of Belarus according to published procedures [1]) were preliminarily dissolved in 10 % ethanol (stock solution with a concentration of 5 mg/mL) and, immediately before the study, put on a support medium for cell culture until the required concentration was reached.

Human seasonal respiratory alpha coronavirus 229E (HCoV-229E) was used. A monolayer cell culture (Vero E6) was grown in the wells of panels (Costar), then the growth medium was removed and cells were infected with 0.01–0.001 $TCID_{50}$/cell of the virus by diluting the virus-containing suspension in a volume of 0.1 mL for 1 hour at 37 °C. The fluid was then removed and the cells were covered with support medium (DMEM) containing various concentrations of test substances. The panels were incubated in an atmosphere containing 5 % CO_2 at 37 °C for 48 h, and morphological changes in the cell monolayer were recorded (cytopathic effect of the virus, an increase ×80). The virus titer in the presence of the test substances and in the control was calculated as lg $TCID_{50}$ (50 % tissue cytopathogenic infectious dose).

The presence of differences in the titer of the virus in comparison with the control was considered as the criterion for antiviral activity. The obtained data were processed by methods generally accepted in virology for determining the number of infectious units, Reed and Muench, statistics for small values of n in an ungrouped data series [9; 10]. Concentrations of 50 and 90 % inhibition of viral replication in the presence of the test substance (EC_{50} and EC_{90}) were determined based on probit analysis and weighted linear regression [11]. The ratios of maximum tolerated concentration (MTC)/EC_{50} and MTC/EC_{90} were used as values indicating the breadth of the range of active non-toxic concentrations of the substance. The MTC was defined as the maximum concentration of a substance that did not affect the morphology of an unstained cell culture.

Results and Discussion. The most pronounced antiviral properties were found in compounds of the castasterone family (Table). Castasterone **1a** and epicastasterone **1b** showed generally comparable antiviral activity against the reproduction of HCoV-229E in Vero E6 cell culture with a decrease in virus titer to 1 lg $TCID_{50}$/mL (equivalent to suppression of viral reproduction by 90 %) and more in the concentration range from MTC to 1/4 MTC. The decrease in virus titer in the presence of (22*S*,23*S*)-epicastasterone **1e** was even more pronounced in the concentration range from MTC to 1/32 MTC and reached 3.3 lg $TCID_{50}$/mL. Homocastasterone showed a slight antiviral effect, a decrease in the titer of the virus by 1 lg $TCID_{50}$/mL in the MTC and 1/2 MTC, inhibition of virus reproduction in the presence of (22*S*,23*S*)-homocastasterone **1c** at the same concentrations was 0.46 lg $TCID_{50}$/mL.

Calculated indexes of the inhibitory effect of the studied substances on the reproduction of HCoV-229E

Compound	Concentration*, μM	Virus titer ± Sx, lg $TCID_{50}$/mL	Difference with the control, lg $TCID_{50}$/mL	EC_{50} (I_{95}) EC_{90} (I_{95}), μM	Ratios MTC/EC_{50} MTC/EC_{90} (semi-quantitative assessment)**
Castasterone **1a**	430.4	4.38 ± 0.36	1.72		
	215.2	4.78 ± 0.46	1.32		
	107.6	5.11 ± 0.52	0.99	31.4 (33.1–28.2)	13.7
	53.8	5.45 ± 0.42	0.65		
	26.9	5.64 ± 0.46	0.46		
	13.4	6.10 ± 0.49	0	103.5 (115.0–92.1)	4.1 (+++)
	0	6.10 ± 0.49	–		
Epicastasterone **1b**	430.4	3.30 ± 0.26	2.00	24.3 (59.8–9.9)	17.7
	215.2	3.48 ± 0.34	1.82		
	107.6	4.30 ± 0.53	1.00		
	53.8–13.4	5.30 ± 0.26	0	103.1 (251.9–42.2)	4.2 (+++)
	0	5.30 ± 0.26	–		
Homocastasterone **1c**	208.9	5.0 ± 0.58	1.10	26.1 (135.6–5.0)	8.0
	104.4	5.05 ± 0.56	1.05		
	52.2	5.64 ± 0.46	0.46		
	26.1–13.1	6.18 ± 0.26	–0.08	140.2 (726.8–26.9)	1.5 (++)
	0	6.10 ± 0.49	–		
(22*S*,23*S*)-Epicastasterone **1e**	1721.5	<2.0	3.30	21.1 (22.4–19.6)	81.6
	860.8	<2.0	3.30		
	430.4	2.48 ± 0.34	2.82	93.6 (100.1–87.6)	18.3 (++++)
	215.2–107.6	4.11 ± 0.52	1.19		
	53.8	4.30 ± 0.32	1.00		
	26.9	5.00 ± 0.53	0.30		
	13.4	5.30 ± 0.26	0		
	0	5.30 ± 0.26	–		

Table ending

Compound	Concentration*, μM	Virus titer ± Sx, lg $TCID_{50}$/mL	Difference with the control, lg $TCID_{50}$/mL	EC_{50} (I_{95}) EC_{90} (I_{95}), μM	Ratios MTC/EC_{50} MTC/EC_{90} (semi-quantitative assessment)**
(22*S*,23*S*)-Homocastasterone **1f**	208.9	5.64 ± 0.46	0.46	75.8 (121.2–47.4)	2.7
	104.4–52.2	5.64 ± 0.46	0.46		
	26.1–13.1	6.18 ± 0.26	–0.08	368.1 (588.7–230.2)	1.1 (+)
	0	6.10 ± 0.49	–		
Epibrassinolide **2b**	52.0	4.30 ± 0.26	1.00	<13.0 (163.1–0.000008) 48.05 (205828–0.01)	>4.0
	26.0	4.37 ± 0.30	0.93		
	13.0	4.48 ± 0.34	0.82		1.1 (++)
	0	5.30 ± 0.26	–		
Homobrassinolide **2c**	50 (101.9) – 6.25(12.6)	6.18 ± 0.26	–0.08	>101.9	<1 (–)
	0	6,10 ± 0,49	–		
(22*S*,23*S*)-Epibrassinolide **2e**	408.4–13.0	5.30 ± 0.26	0	>408.4	<1 (–)
	0	5.30 ± 0.26	–		
(22*S*,23*S*)-Homobrassinolide **2f**	202.1	4.30 ± 0.32	1.80	35.8 (38.4–33.3)	5.6
	101.1	4.30 ± 0.32	1.80		
	50.5	4.64 ± 0.46	1.46		
	25.3	6.18 ± 0.26	–0.08	59.6 (63.9÷ 55.6)	3.4 (+++)
	12.6	6.10 ± 0.49	0		
	0	6.10 ± 0.49	–		

N o t e s: * – the highest concentration corresponds to the MTC; ** – (–) MTC/EC_{90} <1, inactive; (+) MTC/EC_{90} = 1; (++) MTC/EC_{90} = 2, weak activity; (+++) MTC/EC_{90} = 4, medium activity; (++++) MTC/EC_{90} ≥8, high activity [12].

Among brassinolides, epibrassinolide **2b** revealed a noticeable antiviral effect in the concentration range from MTC to 1/4 MTC. The decrease in virus titer compared to the untreated control ranged from 1 to 0.82 lg $TCID_{50}$/mL. (22*S*,23*S*)-Epibrassinolide **2e** and homobrassinolide **2c** were not active, and at the same time, in comparison with them, the virus-inhibiting activity of (22*S*,23*S*)-homobrassinolide **2f** increased significantly, manifesting itself in MTC, 1/2 and 1/4 MTC by a decrease in titer by more than 1 lg $TCID_{50}$/mL.

Conclusions. Studies have shown that BS can have tangible antiviral activity against coronaviruses. (22*S*,23*S*)-Epicastasterone **1e** showed the best results with an EC_{50} value of 21.1 μM. This is quite far from the nanomolar activity of nucleosides, but the very fact of its existence in steroidal phytohormones is interesting and requires explanation. A possible mechanism of the antiviral action of BS may include blocking the initial (fusion) and/or final stages of the interaction of the virus with the cell, as it is the case with betulinic acid derivatives [13–15]. Further research in this direction may be related to the study of the mechanism for the anti-COVID effect of BS, as well as to the study of the properties of their compositions with known antiviral drugs.

Acknowledgement. The work was sponsored by the Belarusian Republican Foundation for Fundamental Research (Agreement no. X21COVID-004).

Благодарности. Работа выполнена при финансовой поддержке Белорусского республиканского фонда фундаментальных исследований (проект X21COVID-004).

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