Received: 28 January 2021 | Accepted: 30 January 2021

DOI: 10.1002/ardp.202100038

FULL PAPER

ARCH PHARM DPhG
Archiv der Pharmazie

(+)-Camphor and (−)-borneol derivatives as potential anti-orthopoxvirus agents

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Funding information
Russian Foundation for Basic Research, Grant/Award Number: 20-33-70067

Abstract

Although the World Health Organisation had announced that smallpox was eradicated over 40 years ago, the disease and other related pathogenic poxviruses such as monkeypox remain potential bioterrorist weapons and could also re-emerge as natural infections. We have previously reported (+)-camphor and (−)-borneol derivatives with an antiviral activity against the vaccinia virus. This virus is similar to the variola virus (VARV), the causative agent of smallpox, but can be studied at BSL-2 facilities. In the present study, we evaluated the antiviral activity of the most potent compounds against VARV, cowpox virus, and ectromelia virus (ECTV). Among the compounds tested, 4-bromo-*N*′-((1*R*,4*R*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)benzohydrazide **18** is the most effective compound against various orthopoxviruses, including VARV, with an EC_{50} value of 13.9 μM and a selectivity index of 206. Also, (+)-camphor thiosemicarbazone **9** was found to be active against VARV and ECTV.

KEYWORDS
cowpox virus, ectromelia virus, vaccinia virus, variola virus, (+)-camphor, (−)-borneol

1 | INTRODUCTION

Forty years ago, smallpox was officially declared eradicated by the World Health Organisation (WHO). Smallpox has struck humanity throughout its history. In the 20th century alone, smallpox outbreaks resulted in the death of about 400 million people. Therefore, the eradication of smallpox was a landmark event. Moreover, it is still the only case in human history when the world's community managed to implement a program for the global eradication of a virus, thereby freeing itself from a severe infection. Variola virus (VARV), the causative agent of smallpox, belongs to the genus *Orthopoxvirus*, with this genus also including the cowpox virus (CPV), monkeypox virus, camelpox virus, ectromelia virus (ECTV), and vaccinia virus (VV), among others. To date, VARV strains have only been kept in two repositories: the State Research Centre for Virology and Biotechnology VECTOR, Koltsovo, Russian Federation, and the Centers for Disease Control and Prevention (CDC), Atlanta, United States. There are no known zoonotic hosts of VARV, whereas other orthopoxviruses (OPVs) may be transmitted to humans by animal hosts. In 2017 and 2018, human monkeypox outbreaks were reported in the Democratic Republic of Congo, Central African Republic, Cameroon, Republic of Congo, Liberia, and Nigeria.[1] From 1970 to 2018, the total number of suspected monkeypox cases was 24,399.[2] The increasing number of zoonotic OPV infections, the potential of using OPVs as a bioterrorism weapon, and the weak vaccination coverage of the population (by 1984, all countries had ceased vaccinating) are all potential threats requiring effective anti-poxvirus agents to be developed.

OPVs are large, linear, double-stranded DNA viruses that replicate using several virus-encoded enzymes. The complex replication mechanism of OPVs provides a number of targets for drug intervention. To date, several compounds have been identified to be effective both in vitro and in vivo. Isatin β-thiosemicarbazone **1** (Figure 1, methisazone, Marboran®) was the first compound identified to possess a marked antiviral activity in mice infected with the smallpox virus.[3] To date, however, there has been limited clinical experience with methisazone in humans.[4] Methisazone is thought to inhibit the transcription process, but the mechanism is incompletely understood. Currently, CMX001 and ST-246 appear to have the greatest potential to be approved for the treatment of OPV infections. The possible target of nucleoside analog CMX001 is the viral DNA polymerase. It has been shown in vitro that the inhibitory concentration of CMX001 against VARV is 0.1 μM, which varies from 0.5 to 0.9 μM against CPV, VV, mousepox, and rabbitpox.[5] The molecular target of ST-246 is the viral protein p37, required to produce the extracellular virus envelope.[6] ST-246 is active against many OPVs, including VARV, monkeypox virus, camelpox virus, and mousepox virus. A team of scientists from the Novosibirsk Institute of Organic Chemistry and the State Research Center VECTOR has developed a structural analog of the drug ST-246: the agent NIOCH-14. This compound is effective against OPVs in both in vivo and in vitro experiments.[7] The mechanism of action of this antiviral agent is the same as that of ST-246, as NIOCH-14 is a pro-drug and is converted into its active metabolite ST-246 in mammals. Despite CMX001, ST-246, and NIOCH-14 being promising candidates, there is a need to further develop antiviral agents against OPVs.

Monoterpenoid derivatives have been extensively studied as therapeutic agents against the proliferation of cancer cells, for treating neurodegenerative disorders, and against virus replication and bacterial infections.[8] Our research group focused on the synthesis of camphor and borneol derivatives reported to be inhibitors of the influenza virus,[9] filoviruses,[10] and OPVs. In particular, we evaluated libraries of compounds for the ability to interfere with the replication of VV in vitro. This virus is similar to the VARV but can be studied at BSL-2 facilities. Over 300 monoterpenoid derivatives were screened, among which esters **1**–**4** based on (−)-borneol, amides **5**–**8** with N-containing heterocycles,[11] the (+)-camphor thiosemicarbazone derivatives **9**–**14**,[12] and (+)-camphor-based *N*-acylhydrazones **15**–**18**[13] were found as good inhibitors of the VV (Figure 2). Derivatives **1**–**18** had IC_{50} (the 50% inhibitory concentration) values in the range of 2.5–70 μM against VV and CC_{50} (the 50% cytotoxic concentration) values in the range of 120–1430 μM. On the basis of the antiviral data obtained, derivatives **1**–**18** were identified as promising OPV inhibitors, which may be candidates for further optimization. As the genus *Orthopoxvirus* includes several virus species pathogenic for humans, an important aspect in the development of OPV inhibitors is the extent of drug action. Therefore, in the present work, we evaluated the antiviral activity of monoterpenoid derivatives **1**–**18** against the different OPVs, including the VARV known to be highly pathogenic for humans.

2 | RESULTS AND DISCUSSION

To study the effect of derivatives **1**–**18** on various OPVs replication, we determined antiviral activity against zoonotic viruses CPV and ECTV and the exclusively anthroponotic agent VARV. The results are shown in Table 1. Among esters **1**–**4**, only derivative **2** demonstrated moderate antiviral activity against VARV and ECTV with an IC_{50} value of 38.0 and 47.7 μM, respectively, and low cytotoxicity with a CC_{50} value of 413.0 μM (selectivity index [SI] equal to 11 and 9, respectively). Esters **1** and **4** are devoid of inhibitory activity against VARV, ECTV, and CPV. Ester **3**, different from ester **2** by an additional methylene group, showed a similar IC_{50} value but was significantly more toxic. Among amides **5**–**8**, only derivative **8** bearing a 4-methylpiperidine fragment demonstrated inhibitory activity against VARV with an IC_{50} value of 14.5 μM (SI = 16). An accordance of antiviral activity was found against VV and VARV in the series of compounds **1**–**8**. Compounds **2** and **8**, showing the low IC_{50} value against VV (3.5 and 2.5 μM respectively), also possessed moderate antiviral activity against VARV. However, in the series, no compound was found with satisfactory antiviral activity against all viruses tested (VV, VARV, CPV, and ECTV).

An extended study of the anti-OPV activity of camphor thiosemicarbazone **9** and its derivatives **10**–**14** indicated thiosemicarbazone **9** to possess inhibitory activity against VARV, with an IC_{50} value of 8.8 μM, along with an SI of 26, and against ECTV, with an

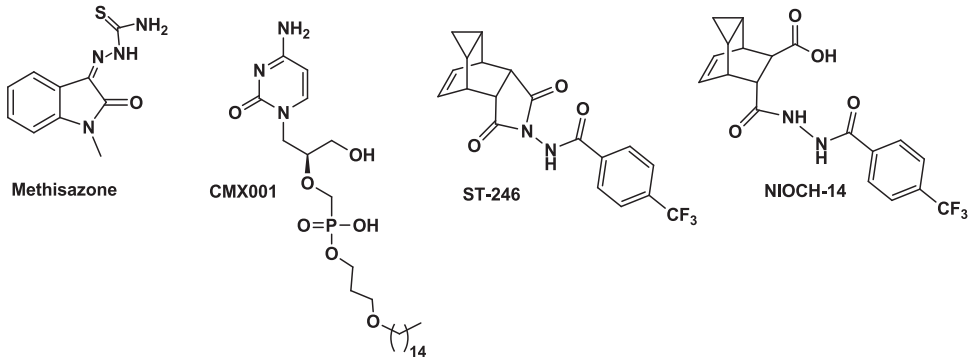


FIGURE 1 Chemical structures of inhibitors of orthopoxvirus replication

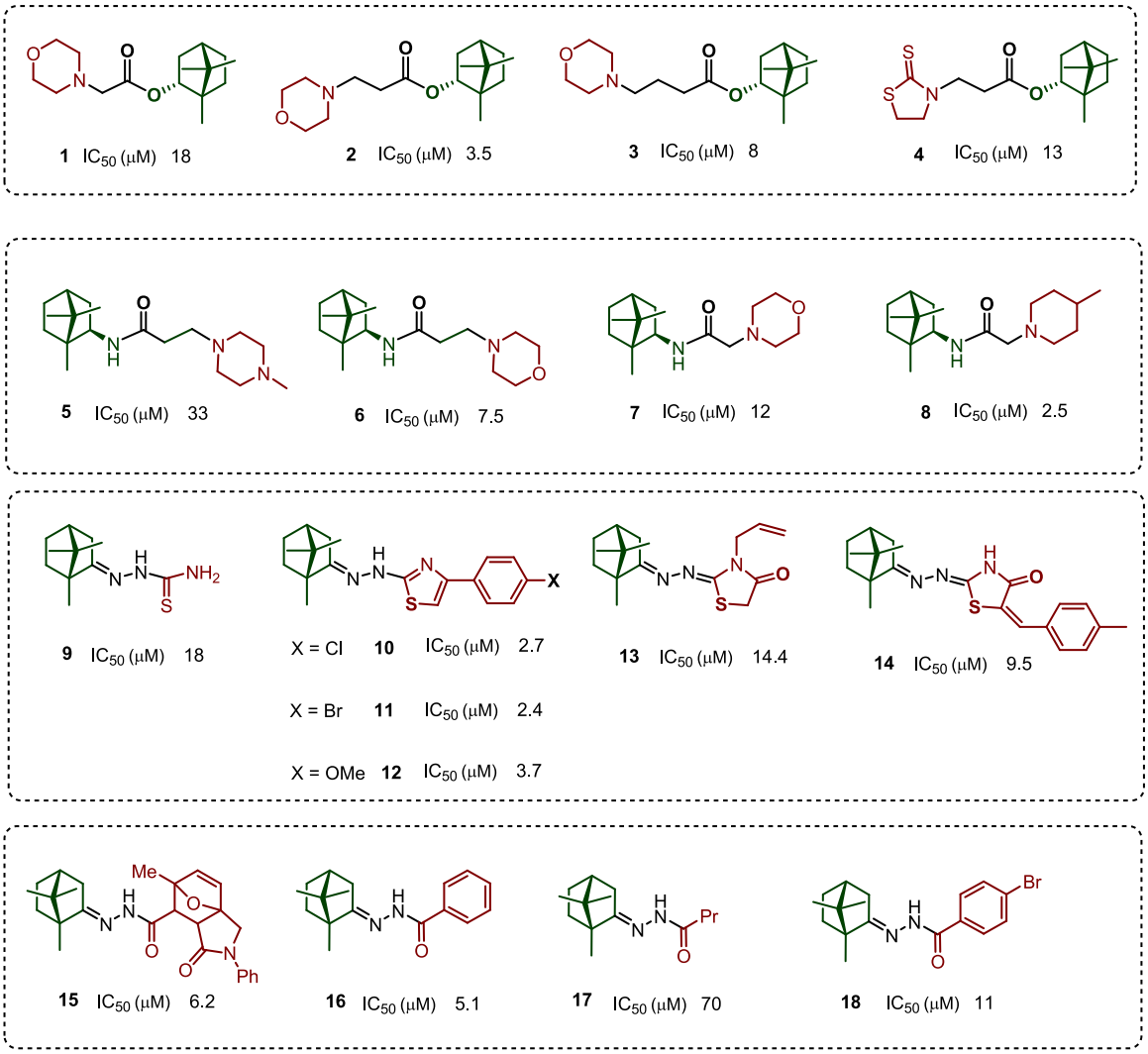


FIGURE 2 Structure and antiviral activity against vaccinia virus of derivatives **1**–**18**. IC_{50}: 50% inhibitory concentration, ensuring 50% cell survival in an infected monolayer

IC_{50} value of 12.4 μM (SI = 19). Transformation of the =N–NH–C(S)–NH_2 group in the thiazole group led to an increased toxicity and decreased antiviral potency. Thus, thiazole **11** displayed an IC_{50} value similar to that of compound **9**, whereas the toxicity was almost threefold higher than the CC_{50} value of 66.8 and 229.0 μM, respectively. Thiazoles **10** and **12** demonstrated low antiviral potency (IC_{50} = 14.7 and 10.3 μM) against VARV and higher cytotoxicity (CC_{50} = 75.9 and 137.6 μM) than thiosemicarbazone **9**. Thiazolidinones **13** and **14** were found not to be active against VARV, CPV, and ECTV.

Among the series of acylhydrazones **15**–**18**, aroyl hydrazones **16** and **18** showed a good inhibitory activity against VARV, CPV, and ECTV. Moreover, derivatives **16** and **18** demonstrated low cytotoxicity and high values of SI. The antiviral activity of compound **18** against CPV and ECTV with SI values of 460 and 854, respectively, is worth noting. Compound **15**, bearing an epoxyisoindole moiety, showed moderate inhibition of OPV replication, with IC_{50} values in the range of 13–29 μM and low cytotoxicity. Compound **17**, with a propyl substituent, turned out to be inactive against the OPVs tested. Also, acylhydrazone **17** can be seen as being inactive against VV (IC_{50} = 70 μM; Figure 2).

In general, there was good agreement with the IC_{50} values for a compound between the VV and the VARV assays, as shown in Figure 2 and Table 1. However, discrepancies were observed for derivatives **4**, **7**, **13**, and **14** that showed an antiviral activity against VV with IC_{50} 13, 12, 14.4, and 9.5 μM, respectively, and were not active in the antiviral assay toward VARV, which is not surprising as VARV and VV differ in their biological properties. It may also be noted that the antiviral activity of the studied compounds against CPV is generally lower than against VV, VARV, and ECTV. This result may be due to different amino acid sequences in the domains of conserved proteins, which could provide different sensitivities of OPVs to the chemical compounds.[15]

As the camphor thiosemicarbazone **9** displayed an inhibitory activity against VARV and ECTV, and =N–NH–C(S)–NH_2 group allows for chemical modifications, we implemented structural modifications on

TABLE 1 Cytotoxicity and anti-orthopoxviruses activities of (+)-camphor and (−)-borneol derivatives **1**–**18** in Vero cells

Compound	CC_{50}[a], μM	IC_{50VARV}[b], μM (SI[e])	IC_{50CPV}[c], μM (SI[e])	IC_{50ECTV}[d], μM (SI[e])
1	363.6 ± 75.0	166.0 ± 77.9 (2)	NA	168.8 ± 79.2 (2)
2	413.0 ± 81.2	38.0 ± 6.8 (11)	145.6 ± 104.9 (3)	47.7 ± 8.5 (9)
3	139.6 ± 71.4	38.5 ± 6.9 (4)	NA	NA
4	319.4 ± 75.1	NA	NA	NA
5	331.4 ± 68.0	215.1 ± 34.3 (2)	177.6 ± 76.1 (2)	42.6 ± 6.8 (8)
6	339.3 ± 70.3	125.1 ± 20.2 (3)	130.8 ± 47.2 (3)	44.2 ± 7.1 (8)
7	396.2 ± 74.5	NA	NA	NA
8	135.4 ± 75.2	14.5 ± 7.9 (16)	62.2 ± 21.5 (2)	33.2 ± 18.1 (4)
9	229.0 ± 53.3	8.8 ± 6.0 (26)	49.3 ± 7.1 (5)	12.4 ± 8.9 (19)
10	75.9 ± 37.0	14.7 ± 10.5 (5)	NA	NA
11	66.8 ± 31.9	3.1 ± 1.0 (11)	NA	15.1 ± 4.7 (4)
12	137.6 ± 25.6	10.3 ± 1.8 (13)	NA	42.8 ± 7.6 (3)
13	50.7 ± 31.1	NA	NA	NA
14	147.8 ± 27.8	59.2 ± 10.5 (2)	NA	NA
15	476.5 ± 71.5	26.5 ± 9.3 (18)	28.8 ± 14.5 (17)	13.1 ± 4.6 (37)
16	1638.5 ± 466.0	21.3 ± 7.5 (76)	14.4 ± 5.2 (117)	10.4 ± 3.7 (157)
17	853.8 ± 206.4	271.2 ± 96.4 (3)	NA	NA
18	2904.9 ± 49.9	13.9 ± 2.4 (206)	6.3 ± 1.7 (460)	3.4 ± 0.6 (854)
Cidofovir	923.0 ± 321.6	29.4 ± 13.9 (31)	89.1 ± 11.8 (10)	23.3 ± 11.1 (40)

Note: CC_{50}, IC_{50VARV}, IC_{50CPV}, and IC_{50ECTV} are presented as $M \pm SD$, where M is the mean and SD is the standard deviation; $n = 3$ is the number of measurements of CC_{50}, IC_{50VARV}, IC_{50CPV}, and IC_{50ECTV}.

[a]CC_{50} is the cytotoxic concentration of a compound causing 50% cell death in an uninfected monolayer.

[b]IC_{50VARV} is the concentration of a compound ensuring 50% cell survival in a monolayer infected with VARV.

[c]IC_{50CPV} is the concentration of a compound ensuring 50% cell survival in a monolayer infected with CPV.

[d]IC_{50ECTV} is the concentration of a compound ensuring 50% cell survival in a monolayer infected with ECTV.

[e]SI is the drug selectivity index (CC_{50}/IC_{50}). Compounds with SI <8 are considered practically inactive according to the recommendations.[14]

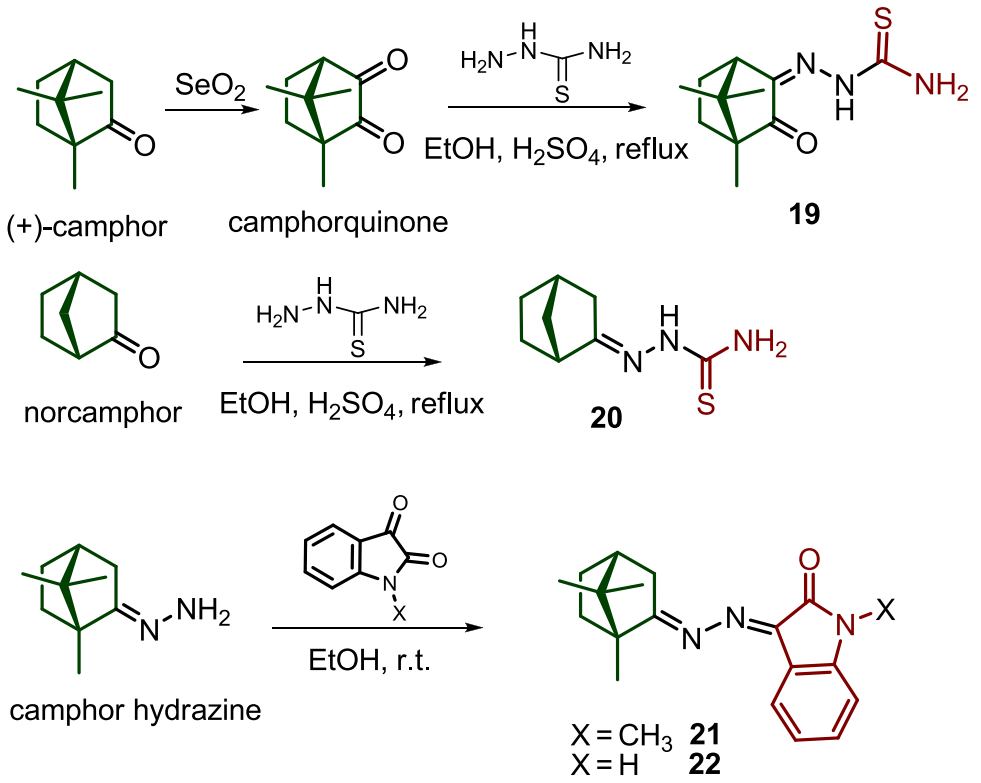
compound **9** to possibly improve toxicity and potency by adding an isatin fragment and modifying the 1,7,7-trimethylbicyclo[2.2.1]heptane region, as outlined in Scheme 1. To study the effect of the 1,7,7-trimethylbicyclo[2.2.1]heptane scaffold on antiviral activity, camphorquinone and norcamphor were coupled with thiosemicarbazide to provide compounds **19** and **20**. The conjugates of camphor hydrazine and isatin **21** or 1-methylisatin **22** were prepared, because the isatin nucleus could be considered as a privileged scaffold for designing biologically active agents.[16] Moreover, it is the constitutive fragment of the anti-OPV drug methisazone (Figure 1).

The synthesized compounds were tested in the preliminary antiviral study against VV. The results are summarized in Table 2. The commercially available agents cidofovir and methisazone were used as a positive control.

Biological evaluation of the thiosemicarbazones of camphorquinone **19** and norcamphor **20** against VV indicated a reduced activity as compared with the camphor thiosemicarbazone **9** (IC_{50} = 18 μM; Figure 2). At the same time, thiosemicarbazones **19** and **20** exhibited lower toxicity (CC_{50} values of 460.4 and 544.0 μM, respectively) than thiosemicarbazone **9** (CC_{50} = 229.0 μM). The conjugation of camphor hydrazone with isatin scaffolds decreased the inhibitory activity against VV and, according to the CC_{50} value, compounds **21** and **22** were found to be quite cytotoxic (CC_{50} values of 16.8 and 35 μM, respectively).

3 | CONCLUSION

In conclusion, we have screened a series of (+)-camphor and (−)-borneol derivatives against different OPV infections. The SAR investigation indicated the 1,7,7-trimethylbicyclo[2.2.1]heptane core structure, *N*-acylhydrazone motif, and thiosemicarbazone group to be favorable for the anti-OPV activity. Among the compounds tested, we have identified acylhydrazones **16** and **18** with a broad-spectrum activity (SI values of 76, 117, and 157; 206, 460, and 854, respectively) against various OPVs, including VARV, CPV, and ECTV.



SCHEME 1 Synthesis of the derivatives **19**–**21**

Several other compounds show promise, including camphor thiosemicarbazone **9** with an IC_{50VARV} value of 8.8 μM and high SI of 26. The transformation of the =N–NH–C(S)–NH_2 group in compound **9** to thiazole or thiazolidin-4-one cycle in compounds **10**–**14** led to a loss of anti-OPV activity. Also, conjugation of the isatin scaffold with 1,7,7-trimethylbicyclo[2.2.1]heptane via a hydrazine linker resulted in a marked increase in cytotoxicity and loss of anti-OPV activity. However, the results indicate that monoterpenoids with the 1,7,7-trimethylbicyclo[2.2.1]heptane core could be a potential scaffold for developing compounds for anti-orthopoxviral therapy.

TABLE 2 The antiviral activities of compounds **19**–**22** against vaccinia virus

Compound	CC_{50}, μM[a]	IC_{50}, μM[b]	SI[c]
19	460.4 ± 156.7	171.7 ± 53.0	2
20	544.0 ± 132.6	212.8 ± 64.9	2
21	16.8 ± 8.7	NA	
22	34.9 ± 16.3	NA	
Cidofovir	923.0 ± 321.6	37.9 ± 18.9	24
Methisazone	192.5 ± 8.9	8.1 ± 4.7	23

Note: Data are presented as *M* ± *SD*, where *M* is the mean and *SD* is the standard deviation; *n* = 3 is the number of CC_{50} and IC_{50} measurements.

[a]CC_{50} is the cytotoxic concentration causing 50% cell death in an uninfected monolayer.

[b]IC_{50} is the concentration ensuring 50% cell survival in a monolayer infected with VV.

[c]SI is the drug selectivity index (CC_{50}/IC_{50}). Compounds with SI <8 are considered practically inactive in accordance with the recommendations.[14]

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

The chemical compounds library contains 18 agents (**1**–**18**) that were screened against OPVs including VARV and four agents (**19**–**22**) that were tested against VV. The synthesis of compounds **1** and **2**,[17] **3**, **5**–**8**,[11] **4**,[18] **9**–**14**,[12] and **15**–**18**[13] was previously described. For the synthesis of derivatives **19**, **21**, and **22**, camphorquinone[19] and camphor hydrazine[13] were prepared according to the procedure reported in the literature from (+)-camphor. Reagents and solvents were purchased from commercial suppliers and used as received. ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded with a Bruker AV-300 (^1H: 300.13 MHz, ^{13}C: 75.47 MHz), AV-400 (^1H: 400.13 MHz, ^{13}C: 100.78 MHz) in $CDCl_3$; chemical shifts δ are expressed in ppm, relative to residual [$\delta(CHCl_3)$ 7.24, $\delta(CDCl_3)$ 76.90]. Elemental analysis was carried out using a Euro EA3000 C, H, N, S analyzer.

The original spectra and the InChI codes of the investigated compounds, together with some biological activity data, are provided as Supporting Information.

4.1.2 | Synthesis of 2-((1*S*,4*R*)-4,7,7-trimethyl-3-oxobicyclo[2.2.1]heptan-2-ylidene)-hydrazinecarbothioamide (**19**)

A solution of thiosemicarbazide (0.01 mol) in ethanol was added to a solution of camphorquinone (0.01 mol) in ethanol and several drops of H_2SO_4. The mixture was heated at reflux for 24 h and then washed with brine and extracted with $CHCl_3$, dried (Na_2SO_4), filtered, and concentrated. The crude product was purified by recrystallization from the mixture hexane/$CHCl_3$ 5:1. Yield: 51%, a yellow solid; mp: 122.6°C; NMR ^{1}H (400 MHz, $CDCl_3$, δ, ppm): 0.82 (3H, s), 0.98 (3H, s), 0.99 (3H, s), 1.41–1.57 (2H, m), 1.73–1.85 (1H, m), 1.99–2.12 (1H, m), 2.97–3.04 (1H, m), 6.85 (1H, br s), 7.54 (1H, br s), 9.21 (1H, br s). NMR ^{13}C (100 MHz, $CDCl_3$, δ, ppm): 204.7 s (C(O)), 180.2 s (C(S)), 152.1 s (C(N)), 58.3 s, 47.4 d, 45.3 s, 30.4 t, 23.8 t, 20.6 q, 17.6 q, 8.9 q. High-resolution electrospray ionization mass spectrometry (HRMS [ESI]) (*m*/*z*): [M^+] calcd. for $C_{11}H_{17}O_1N_3S_1$: 239.1087, found: 239.1089.

4.1.3 | Synthesis of 2-((1*R*,4*S*)-bicyclo[2.2.1]heptan-2-ylidene)hydrazinecarbothioamide (**20**)

A solution of thiosemicarbazide (0.006 mol) in ethanol was added to a solution of norcamphor (0.006 mol) in ethanol and several drops of H_2SO_4. The mixture was heated at reflux in 10 h and then washed with brine and extracted with $CHCl_3$, dried (Na_2SO_4), filtered, and concentrated. The crude product was purified by silica gel column chromatography (eluent: hexane/ethyl acetate). Yield: 45%, a white solid, mp: 73.7°C; NMR ^{1}H (400 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 1.19–1.31 (1H, m), 1.36–1.52 (3H, m), 1.61–1.79 (2H, m), 1.87–1.95 (1H, m), 2.09–2.17 (1H, m), 2.55–2.60 (1H, m), 2.72–2.79 (1H, m), 6.57 (1H, br s), 7.11 (1H, br s), 8.48 (1H, br s). NMR ^{13}C (100 MHz, $CDCl_3$, δ, ppm): 178.3 s (C(S)), 164.3 s (C(N)), 44.2 d, 38.5 t, 35.6 d, 35.4 t, 27.3 t, 26.7 t. HRMS (ESI) (*m*/*z*): [M^+] calcd. for $C_8H_{13}N_3S_1$: 183.0825, found: 183.0823.

4.1.4 | Synthesis of 1-methyl-3-(((1*R*,4*R*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)hydrazono)-indolin-2-one (**21**)

To a solution of camphor hydrazone (3 mmol) in 5 ml of ethanol, 1-methylisatin (3 mmol) was added, and the mixture was stirred at room temperature for 8 h. The mixture was then washed with brine and extracted with $CHCl_3$, dried (Na_2SO_4), filtered, and concentrated. The crude product was purified by silica gel column chromatography (eluent: $CHCl_3$/MeOH) to give a yellow solid. Yield: 54%; mp: 82.3°C; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 0.81 (3H, s), 0.95 (3H, s), 1.19 (3H, s), 1.18–1.26 (1H, m), 1.47–1.54 (1H, m), 1.75–1.88 (2H, m), 1.91–1.93 (1H, m), 1.99–2.06 (1H, m), 2.51–2.59 (1H, m), 3.23 (3H, s), 6.79 (1H, d, *J* = 7.7), 6.98–7.02 (1H, m), 7.32–7.36 (1H, m), 7.91 (1H, d, *J* = 7.7). NMR ^{13}C (100 MHz, $CDCl_3$, δ, ppm): 178.5 s (C(N)), 164.2 s (C(O)), 146.4 s, 145.6 s, 132.3 d (CH–Ar), 128.2 d (CH–Ar), 122.6 d (CH–Ar), 116.7 s, 108.2 d (CH–Ar), 53.3 s, 48.1 s, 43.8 d, 35.6 t, 32.4 t, 26.9 t, 25.8 q, 19.4 q, 18.6 q, 11.1 q. HRMS (ESI) (*m*/*z*): [M^+] calcd. for $C_{19}H_{23}O_1N_3$: 309.1836, found: 309.1838.

4.1.5 | Synthesis of 3-(((1*R*,4*R*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)hydrazono)-indolin-2-one (**22**)

To a solution of camphor hydrazone (3 mmol) in 5 ml of ethanol, isatin (3 mmol) was added, and the mixture was stirred at room temperature for 8 h. The mixture was then washed with brine and extracted with $CHCl_3$, dried (Na_2SO_4), filtered, and concentrated. The crude product was purified by silica gel column chromatography (eluent: $CHCl_3$/MeOH) to give a yellow solid. Yield: 42%; mp: 76.4°C; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 0.84 (3H, s), 0.97 (3H, s), 1.21 (3H, s), 1.22–1.26 (1H, m), 1.48–1.55 (1H, m), 1.78–1.91 (2H, m), 1.94–1.96 (1H, m), 2.03–2.11 (1H, m), 2.57–2.65 (1H, m), 6.89 (1H, d, *J* = 7.8), 6.98–7.02 (1H, m), 7.28–7.33 (1H, m), 7.94 (1H, d, *J* = 7.8), 9.21 (1H, br s). NMR ^{13}C (100 MHz, $CDCl_3$, δ, ppm): 179.1 s (C(N)), 166.1 s (C(O)), 146.9 s, 143.1 s, 132.5 d (CH–Ar), 128.5 d (CH–Ar), 122.7 d (CH–Ar), 117.19 s, 110.6 d (CH–Ar), 53.3 s, 47.8 s, 43.8 d, 35.7 t, 32.5 t, 26.9 t, 19.4 q, 18.6 q, 11.1 q. HRMS (ESI) (*m*/*z*): [M^+] calcd. for $C_{18}H_{21}O_1N_3$: 295.1679, found: 295.1680.

4.2 | Biological assays

4.2.1 | Cells and viruses

Vaccinia virus (VV, strain Copenhagen), cowpox virus (CPV, strain Grishak), mousepox virus—ectromelia (ECTV, strain K-1), and variola virus (VARV, strain Ind-3a), obtained from the state collection of pathogens of viral infections and rickettsioses of SRC VB Vector, were used in the work. Virus-containing suspensions with concentrations ranging from 5.6 to 6.7 $\log_{10}$ PFU/ml were prepared in Vero cell culture medium using these strains. Virus-containing material was packaged in individual tubes and stored at a temperature of −70°C. Vero cell monolayer was grown in Dulbecco's Modified Eagle's medium (DMEM; OJSC BioloT) in the presence of 10% fetal bovine serum (FBS; HyClone) supplemented with penicillin (100 IU/ml) and streptomycin (100 mg/ml). The same medium supplemented with 2% FBS, penicillin (100 IU/ml), and streptomycin (100 mg/ml) was used to support virus cultivation.

4.2.2 | Cytotoxicity assay and determination of anti-OPV activities

All experiments with live VARV were conducted at SRC VB Vector in a maximum containment facility (BSL-4) using insulating

pneumatic suits. Viruses were produced in Vero cell culture in DMEM. The virus concentration in the culture liquid was determined by plaque titration in Vero cell culture, calculated, and expressed in decimal logarithms of plaque-forming units in 1 ml ($\log_{10}$ PFU/ml).[20] The concentration of the virus in the samples used in the work ranged from 5.6 to 6.1 $\log_{10}$ PFU/ml. The series of viruses with the indicated titer was stored and used in work at −70°C.

The antiviral efficacy of the compounds was evaluated as follows. In wells of 96-well plates containing a monolayer of Vero cells in 100 μl of DMEM medium with 2% fetal serum, 50 μl of serial dilutions of the test compounds were first introduced and then 50 μl of a dilution of OPV at a dose of 1000 PFU/well was added.

The toxicity of the compounds was determined by the Vero cell death caused by the drug in the wells of the plate, into which the virus was not introduced. Monolayers of cells were used as controls in the wells of the plate, into which virus without compounds (virus control) and monolayers of cells in wells into which neither the virus nor the compound was introduced (cell culture control) were introduced. After incubation for 4 days, the monolayer of cells was stained with vital dye neutral red for 2 h. After removing the dye and washing the wells from its unbound fraction, a lysis buffer was added. The amount of dye adsorbed by the living cells of the monolayer was evaluated by optical density (OD), which is an indication of the number of cells undisturbed under the influence of the virus in a monolayer. The OD was measured on an EMax spectrophotometer (Molecular Devices) at a wavelength of 490 nm. Results were processed using the Soft Max Pro 4.0 program, which computed the 50% toxic concentration (CC_{50} in μM) and 50% inhibitory concentration (IC_{50} in μM). The SI was determined as $SI = CC_{50}/IC_{50}$ using the corresponding concentrations.

ACKNOWLEDGMENTS

The authors would like to acknowledge the Multi-Access Chemical Service Centre SB RAS for spectral and analytical measurements. This study has been supported by a Russian Foundation for Basic Research (RFBR) Grant 20-33-70067. The experiments with live VARV supported the state assignment of State Research Centre of Virology and Biotechnology VECTOR.

CONFLICTS OF INTERESTS

The authors declare that there are no conflicts of interests.

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REFERENCES

[1] K. Simpson, D. Heymann, C. S. Brown, W. J. Edmunds, J. Elsgaard, P. Fine, H. Hochrein, N. A. Hoff, A. Green, C. Ihekweazu, T. C. Jones, S. Lule, J. Maclennan, A. McCollum, B. Mühlemann, E. Nightingale, D. Ogoina, A. Ogunleye, B. Petersen, J. Powell, O. Quantick, A. W. Rimoin, D. Ulaeato, A. Wapling, *Vaccine* **2020**, *38*, 5077. https://doi.org/10.1016/j.vaccine.2020.04.062

[2] K. N. Durski, A. M. McCollum, Y. Nakazawa, B. W. Petersen, M. G. Reynolds, S. Briand, M. H. Djingarey, V. Olson, I. K. Damon, A. Khalakdina, *Morb. Mortal. Wkly. Rep.* **2018**, *67*, 306. https://doi.org/10.15585/mmwr.mm6710a5

[3] D. J. Bauer, K. R. Dumbell, P. Fox-Hulme, P. W. Sadler, *Bull. W. H. O.* **1962**, *26*, 727.

[4] D. M. McLean, *Clin. Infect. Dis.* **2006**, *42*, 1653. https://doi.org/10.1086/504081

[5] K. Y. Hostetler, *Antiviral Res.* **2009**, *82*, 84. https://doi.org/10.1016/j.antiviral.2009.01.005

[6] G. Yang, D. C. Pevear, M. H. Davies, M. S. Collett, T. Bailey, S. Rippen, L. Barone, C. H. Burns, G. Rhodes, S. Tohan, J. W. Huggins, R. O. Baker, R. L. M. Buller, E. Touchette, K. Waller, J. Schriewer, J. Neyts, E. DeClercq, K. Jones, D. Hruby, R. Jordan, *J. Virol.* **2005**, *79*, 13139. https://doi.org/10.1128/JVI.79.20.13139-13149.2005

[7] O. Y. Mazurkov, A. S. Kabanov, L. N. Shishkina, A. A. Sergeev, M. O. Skarnovich, N. I. Bormotov, M. A. Skarnovich, A. S. Ovchinnikova, K. A. Titova, D. O. Galahova, L. E. Bulychev, A. A. Sergeev, O. S. Taranov, B. A. Selivanov, A. Y. Tikhonov, E. L. Zavjalov, A. P. Agafonov, A. N. Sergeev, *J. Gen. Virol.* **2016**, *97*, 1229. https://doi.org/10.1099/jgv.0.000422

[8] N. F. Salakhutdinov, K. P. Volcho, O. I. Yarovaya, *Pure Appl. Chem.* **2017**, *89*, 1105. https://doi.org/10.1515/pac-2017-0109

[9] A. S. Sokolova, O. I. Yarovaya, A. V. Shernyukov, Y. V. Gatilov, Y. V. Razumova, V. V. Zarubaev, T. S. Tretiak, O. I. Kiselev, N. F. Salakhutdinov, *Eur. J. Med. Chem.* **2015**, *105*, 263. https://doi.org/10.1016/j.ejmech.2015.10.010

[10] A. S. Sokolova, O. I. Yarovaya, A. V. Zybkina, E. D. Mordvinova, N. S. Shcherbakova, A. V. Zaykovskaya, D. S. Baev, T. G. Tolstikova, D. N. Shcherbakov, O. V. Pyankov, R. A. Maksyutov, N. F. Salakhutdinov, *Eur. J. Med. Chem.* **2020**, *207*, 112726. https://doi.org/10.1016/j.ejmech.2020.112726

[11] A. S. Sokolova, O. I. Yarovaya, N. I. Bormotov, L. N. Shishkina, N. F. Salakhutdinov, *Chem. Biodivers.* **2018**, *15*, e180015. https://doi.org/10.1002/cbdv.201800153

[12] A. S. Sokolova, O. I. Yarovaya, N. I. Bormotov, L. N. Shishkina, N. F. Salakhutdinov, *MedChemComm* **2018**, *9*, 1746. https://doi.org/10.1039/C8MD00347E

[13] K. S. Kovaleva, F. I. Zubkov, N. I. Bormotov, R. A. Novikov, P. V. Dorovatovskii, V. N. Khrustalev, Y. V. Gatilov, V. V. Zarubaev, O. I. Yarovaya, L. N. Shishkinad, N. F. Salakhutdinov, *MedChemComm* **2018**, *9*, 2072. https://doi.org/10.1039/C8MD00442K

[14] R. U. Khabriev, in *Guidelines for Experimental (Preclinical) Study of New Pharmacological Substances*, Izdatelstvo Meditsina, Moscow **2005**.

[15] S. Duraffour, R. Snoeck, R. de Vos, J. van Den Oord, J. Crance, D. Garin, D. Hruby, R. Jordan, D. E. Clercq, A. Graciela, *Antivir. Ther.* **2007**, *12*(8), 1205.

[16] C. Melis, R. Meleddu, A. Angeli, S. Distinto, G. Bianco, C. Capasso, F. Cottiglia, R. Angius, C. T. Supuran, E. Maccionia, *J. Enzyme Inhib. Med. Chem.* **2017**, *32*, 68. https://doi.org/10.1080/14756366.2016.1235042

[17] A. Sokolova, O. Yarovaya, M. Semenova, A. Shtro, Y. Orshanskay, V. Zarubaev, N. Salakhutdinov, *MedChemComm* **2017**, *8*, 960. https://doi.org/10.1039/C6MD00657D

[18] A. Sokolova, O. Yarovaya, A. Shtro, M. Borisova, E. Morozova, T. Tolstikova, V. Zarubaev, N. Salakhutdinov, *Chem. Heterocycl. Compd.* **2017**, *53*, 371. https://doi.org/10.1007/s10593-017-2063-3

[19] M. S. Singh, *Phosphorus, Sulfur Silicon Relat. Elem.* **1995**, *106*, 187.

[20] B. Mahy, H. O. Kangro, *Virology Methods Manual*, Academic Press, Cambridge, Massachusetts **1996**.

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How to cite this article: A. S. Sokolova, K. S. Kovaleva, O. I. Yarovaya, N. I. Bormotov, L. N. Shishkina, O. A. Serova, A. A. Sergeev, A. P. Agafonov, R. A. Maksuytov, N. F. Salakhutdinov, *Arch. Pharm.* **2021**, e2100038.