Available online at www.sciencedirect.com

ScienceDirect

Mendeleev Commun., 2023, **33**, 537–538

Mendeleev Communications

New N^1-(4-aryloxybenzyl)uracils containing N^3-positioned 4-(trimethyleneoxy)benzoic acid moiety, and study of their antiviral activity against SARS-CoV-2 and influenza virus

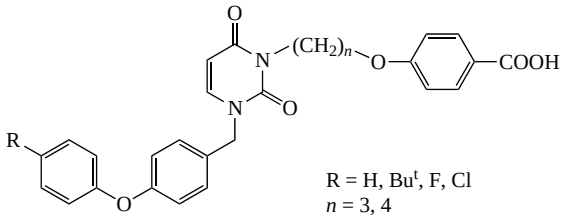
Mikhail S. Novikov,[a] Maria P. Paramonova,[a] Ilia M. Kirillov,[b] Olga A. Smirnova,[c] Anastasia L. Khandazhinskaya,*[c] Alexander O. Chizhov,[d] Sergey N. Kochetkov,[c] Alexander V. Ivanov[c] and Irina F. Fedyakina[b]

[a] *Volgograd State Medical University, 400131 Volgograd, Russian Federation*
[b] *N. F. Gamaleya National Research Center for Epidemiology and Microbiology, 123098 Moscow, Russian Federation*
[c] *V. A. Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, 119991 Moscow, Russian Federation. E-mail: khandazhinskaya@bk.ru*
[d] *N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 119991 Moscow, Russian Federation*

DOI: 10.1016/j.mencom.2023.06.031

New title uracil derivatives, 4-{3-[2,6-dioxo-3-(4-aryloxybenzyl)-3,6-dihydropyrimidin-1(2*H*)-yl]propoxy}benzoic acids and their butoxy homologues, were obtained in three steps using 2,4-bis(trimethylsilyloxy)pyrimidine, 4-aryloxybenzyl bromides and methyl 4-(ω-bromoalkoxy)benzoates as the key reactants. The compounds were studied as inhibitors of H1N1 influenza virus and SARS-CoV-2 replication in MDCK and Vero E6 cell cultures, respectively, which revealed that the tested compounds had high levels of anti-SARS-CoV-2 activity.

Anti-SARS-CoV-2 agents

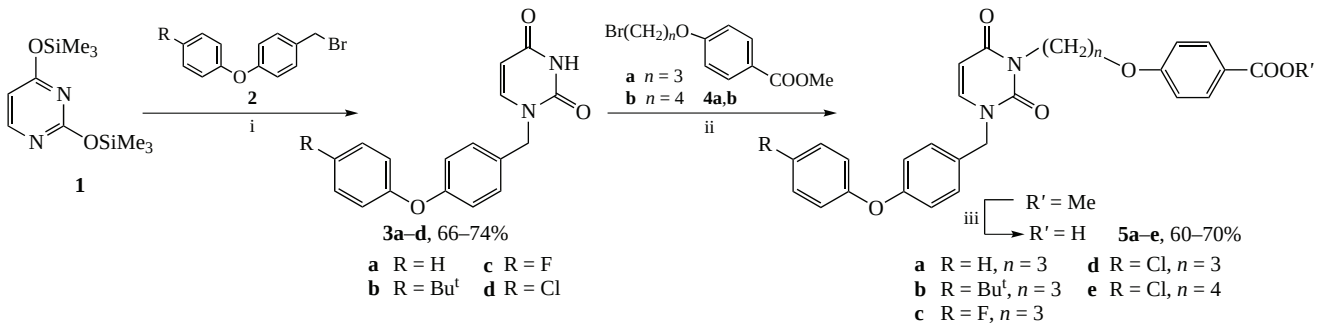


Keywords: uracil derivatives, benzoic acid derivatives, SARS-CoV-2, antiviral activity, *N*-alkylation.

The 2019 global pandemic caused by the SARS-CoV-2 coronavirus continues to spread around the world. As of May 3, 2023, over 765 million people have been infected and 6.9 million have already died.[1] The SARS-CoV-2 genome is a single-stranded positively charged RNA.[2] Like other coronaviruses, SARS-CoV-2 synthesizes various viral enzymes and proteins necessary for virus entry, replication, and pathogenesis, including structural and non-structural proteins that can be used as targets for chemotherapeutic effects.[3] SARS-CoV-2 is highly pathogenic in the elderly and those with high risk factors and can develop into severe, life-threatening acute respiratory distress syndrome.[4] Several anti-SARS-CoV-2 drugs (remdesivir, molnupiravir and nirmatrelvir) have shown clinical efficacy in the early stages of COVID-19 infection.[5–7] However, as new variants of the virus with different mutations in the genome appear, resistance to antiviral drugs develops. For this reason, the search for new, more effective inhibitors of SARS-CoV-2 replication is relevant.[8,9]

Some uracil derivatives of non-nucleoside type have previously been found to inhibit viral polymerases. These compounds inhibit HCMV,[10,11] VZV,[10,11] HIV[12,13] and HCV[14] replication. Recently, we have found a moderate activity of N^1,N^3-disubstituted uracil derivatives against SARS-CoV-2 *in vitro*.[15] In this work, we synthesized new uracil derivatives and evaluated their antiviral activity against SARS-CoV-2 *in vitro* in order to determine their potential for further study as new antiviral agents blocking SARS-CoV-2 infection.

The synthesis of the target compounds (Scheme 1) was started with the reflux of 2,4-bis(trimethylsilyloxy)pyrimidine **1** with benzylic bromides **2** in 1,2-dichloroethane for 24 h, as described previously,[10,13] to afford 1-(4-aryloxybenzyl)uracil



Scheme 1 *Reagents and conditions*: i, 1,2-dichloroethane, Δ, 24 h; ii, K_2CO_3, DMF, 80 °C; iii, NaOH, EtOH–H_2O, then HCl (aq.).

Table 1 Anti-SARS-CoV-2 activity of compounds **5a–e** in cell culture Vero E6.

Compound	EC_{50}/μM[a]	EC_{90}/μM	EC_{99}/μM	CC_{50}/μM[b]	SI[c]
5a	0.016	0.039	0.145	>50	>3125
5b	0.017	0.040	0.133	>50	>2941
5c	0.018	0.043	0.145	>50	>2778
5d	0.016	0.041	0.163	>50	>3125
5e	0.148	0.220	0.387	>50	>338
Molnupiravir	0.3[18]	–	–	>50	>167
4-*N*-Hydroxy-cytidine	5.3[19]	–	–	>100	>19

[a] The effective concentration required to reduce the formation of viral plaques by 50%. [b] Cytotoxic concentration required to reduce cell growth by 50%. [c] Selectivity index, ratio CC_{50}/EC_{50}.

derivatives **3a**–**d**. These derivatives were then treated with methyl 4-(ω-bromoalkoxy)benzoates **4a**,**b**. The thus formed intermediate methyl esters of acids **5** (R′ = Me) were subjected to alkaline hydrolysis,[13] which upon acidification liberated the corresponding target 4-substituted benzoic acids **5a**–**e** (R′ = H, see Scheme 1).

The antiviral activity of compounds **5a**–**e** against SARS-CoV-2 was determined in the system of infected green monkey kidney cells Vero E6, one of the most permissive cell lines.[16] The cells were infected with the virus at a low multiplicity of infection (MOI 0.1) by incubation with virions for 2 h, after which the culture medium was replaced with a fresh one without virus, but containing substances at a concentration 10 μM. The levels of virus replication were determined by measuring the number of virions in a conditioned medium by detecting SARS-CoV-2 genomic RNA by reverse transcription and real-time PCR.

The results presented in Table 1 show that all tested compounds exhibited a high level of anti-SARS-CoV-2 activity, significantly exceeding the anti-SARS agents such as molnupiravir and 4-*N*-hydroxycytidine. These compounds did not have a toxic effect on Vero E6 cells at concentrations up to 50 μM. Compounds **5a**–**d** showed the similar level of suppression of SARS-CoV-2 replication in Vero E6 cell culture. At the same time, compound **5e** turned out to be an order of magnitude less active than its lower homologue **5d**. Apparently, the length of the polymethylene bridge connecting uracil and benzoic acid residues is of great importance in establishing anti-SARS-CoV-2 activity, and elongation of the bridge from three methylene units to four leads to a decrease in antiviral properties.

In addition, the compounds have been investigated as potential inhibitors of influenza virus replication. This was done in a culture of MDCK cells infected with influenza A virus strain A/California/7/2009 (H1N1)pdm09, followed by activity determination by analysis of its cytopathogenic effect.[17] However, none of the substances showed appreciable activity at concentrations up to 50 μM (data not provided).

In conclusion, the synthesis of new uracil-containing derivatives of benzoic acids was carried out, and the compounds of this series showed a high level of anti-SARS-CoV-2 activity *in vitro*. The results obtained can serve as the basis for the creation of a drug for the treatment and prevention of coronavirus infections.

The work was supported by the state task ‘Nucleic bases derivatives as inhibitors of SARS-CoV-2 replication’.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2023.06.031.

References

1 *WHO Coronavirus (COVID-19) Dashboard*, https://covid19.who.int.
2 P. V’kovski, A. Kratzel, S. Steiner, H. Stalder and V. Thiel, *Nat. Rev. Microbiol*., 2021, **19**, 155.
3 R. Yadav, J. K. Chaudhary, N. Jain, P. K. Chaudhary, S. Khanra, P. Dhamija, A. Sharma, A. Kumar and S. Handu, *Cells*, 2021, **10**, 821.
4 M. Gilis, N. Chagrot, S. Koeberle, T. Tannou, A.-S. Brunel, C. Chirouze and K. Bouiller, *J. Med. Virol*., 2021, **93**, 2453.
5 R. L. Gottlieb, C. E. Vaca, R. Paredes, J. Mera, B. J. Webb, G. Perez, G. Oguchi, P. Ryan, B. U. Nielsen, M. Brown, A. Hidalgo, Y. Sachdeva, S. Mittal, O. Osiyemi, J. Skarbinski, K. Juneja, R. H. Hyland, D. Phil, A. Osinusi, S. Chen, G. Camus, M. Abdelghany, S. Davies, N. Behenna-Renton, F. Duff, F. M. Marty, M. J. Katz, A. A. Ginde, S. M. Brown, J. T. Schiffer and J. A. Hill, *N. Engl. J. Med*., 2022, **386**, 305.
6 A. Jayk Bernal, M. M. Gomes da Silva, D. B. Musungaie, E. Kovalchuk, A. Gonzalez, V. Delos Reyes, A. Martín-Quirós, Y. Caraco, A. Williams-Diaz, M. L. Brown, J. Du, A. Pedley, C. Assaid, J. Strizki, J. A. Grobler, H. H. Shamsuddin, R. Tipping, H. Wan, A. Paschke, J. R. Butterton, M. G. Johnson and C. De Anda, *N. Engl. J. Med*., 2022, **386**, 509.
7 J. Hammond, H. Leister-Tebbe, A. Gardner, P. Abreu, W. Bao, W. Wisemandle, M. L. Baniecki, V. M. Hendrick, B. Damle, A. Simón-Campos, R. Pypstra and J. M. Rusnak, *N. Engl. J. Med*., 2022, **386**, 1397.
8 A. M. Szemiel, A. Merits, R. J. Orton, O. A. MacLean, R. M. Pinto, A. Wickenhagen, G. Lieber, M. L. Turnbull, S. Wang, W. Furnon, N. M. Suarez, D. Mair, A. da Silva Filipe, B. J. Willett, S. J. Wilson, A. H. Patel, E. C. Thomson, M. Palmarini, A. Kohl and M. E. Stewart, *PLoS Pathog*., 2021, **17**, e1009929.
9 A. K. Padhi and T. Tripathi, *Biochem. Biophys. Res. Commun*., 2022, **629**, 54.
10 M. P. Paramonova, A. A. Ozerov, A. O. Chizhov, R. Snoeck, G. Andrei, A. L. Khandazhinskaya and M. S. Novikov, *Mendeleev Commun*., 2019, **29**, 638.
11 M. P. Paramonova, R. Snoeck, G. Andrei, A. L. Khandazhinskaya and M. S. Novikov, *Mendeleev Commun*., 2020, **30**, 602.
12 D. A. Babkov, A. L. Khandazhinskaya, A. O. Chizhov, G. Andrei, R. Snoeck, K. L. Seley-Radtke and M. S. Novikov, *Bioorg. Med. Chem*., 2015, **23**, 7035.
13 M. S. Novikov, O. N. Ivanova, A. V. Ivanov, A. A. Ozerov, V. T. Valuev-Elliston, K. Temburnikar, G. V. Gurskaya, S. N. Kochetkov, C. Pannecouque, J. Balzarini and K. L. Seley-Radtke, *Bioorg. Med. Chem*., 2011, **19**, 5794.
14 A. Magri, A. A. Ozerov, V. L. Tunitskaya, V. T. Valuev-Elliston, A. Wahid, M. Pirisi, P. Simmonds, A. V. Ivanov, M. S. Novikov and A. H. Patel, *Sci. Rep*., 2016, **6**, 29487.
15 A. E. Siniavin, M. S. Novikov, V. A. Gushchin, A. A. Terechov, I. A. Ivanov, M. P. Paramonova, E. S. Gureeva, L. I. Russu, N. A. Kuznetsova, E. V. Shidlovskaya, S. I. Luyksaar, D. V. Vasina, S. A. Zolotov, N. A. Zigangirova, D. Y. Logunov and A. L. Gintsburg, *Int. J. Mol. Sci*., 2022, **23**, 10171.
16 D. A. Babkov, V. T. Valuev-Elliston, M. P. Paramonova, A. A. Ozerov, A. V. Ivanov, A. O. Chizhov, A. L. Khandazhinskaya, S. N. Kochetkov, J. Balzarini, D. Daelemans, C. Pannecouque, K. L. Seley-Radtke and M. S. Novikov, *Bioorg. Med. Chem*., 2015, **23**, 1069.
17 *WHO Manual on Animal Influenza Diagnosis and Surveillance*, 18–23 August, 2004, Hokkaido University, Sapporo, Japan.
18 T. P. Sheahan, A. C. Sims, S. Zhou, R. L. Graham, A. J. Pruijssers, M. L. Agostini, S. R. Leist, A. Schäfer, K. H. Dinnon 3rd, L. J. Stevens, J. D. Chappell, X. Lu, T. M. Hughes, A. S, George, C. S. Hill, S. A. Montgomery, A. J. Brown, G. R. Bluemling, M. G. Natchus, M. Saindane, A. A. Kolykhalov, G. Painter, J. Harcourt, A. Tamin, N. J. Thornburg, R. Swanstrom, M. R. Denison and R. S. Baric, *Sci. Transl. Med*., 2020, **12**, eabb5883.
19 E. S. Matyugina, M. S. Novikov, L. I. Kozlovskaya, V. P. Volok, E. Y. Shustova, A. A. Ishmukhametov, S. N. Kochetkov and A. L. Khandazhinskaya, *Acta Naturae*, 2021, **13**, 78.

Received: 18th November 2022; Com. 22/7045