Mendeleev Communications

Mendeleev Commun., 2025, **35**, 606–608

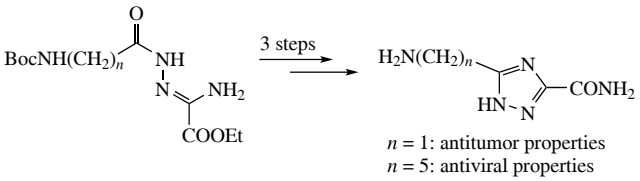
5-Amino-1,2,4-triazole-3-carboxamide homologues and their biological potential

Eugenia S. Oleynik,*[a] Anna A. Shmarina,[a] Ekaterina R. Mitina,[a] Ekaterina A. Mikhina,[a] Ilya A. Semenov,[a] Ekaterina D. Savina,[a,b] Lyubov E. Grebenkina,[a] Ekaterina M. Zhidkova,[b] Ekaterina A. Lesovaya,[b,c,d] Elizaveta N. Vetrova,[e] Olga N. Sineva[f] and Andrey V. Matveev[a]

[a] *M. V. Lomonosov Institute of Fine Chemical Technologies, MIREA – Russian Technological University, 119571 Moscow, Russian Federation. E-mail: oleynik_evgenia@mail.ru*
[b] *N. N. Blokhin National Medical Research Center of Oncology, 115478 Moscow, Russian Federation*
[c] *Peoples' Friendship University of Russia (RUDN University), 117198 Moscow, Russian Federation*
[d] *I. P. Pavlov Ryazan State Medical University, 390026 Ryazan, Russian Federation*
[e] *N. F. Gamaleya National Research Center of Epidemiology and Microbiology, 123098 Moscow, Russian Federation*
[f] *G. F. Gause Institute of New Antibiotics, 119021 Moscow, Russian Federation*

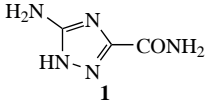
DOI: 10.71267/mencom.7753

New homologues of 5-amino-1,2,4-triazole-3-carboxamide, *viz*., 5-(ω-aminoalkyl)-1,2,4-triazole-3-carboxamides have been synthesized. The intermediate acyloxalamidrazones undergo thermal cyclization to 1,2,4-triazoles with the better yield in the absence of catalyst while in the presence of 10 mol% TsOH the main product is the corresponding 1,3,4-oxadiazole derivative. The obtained compounds have demonstrated various biological properties comparable to those of the nucleoside drug ribavirin.



Keywords: 5-(ω-aminoalkyl)-1,2,4-triazole-3-carboxamides, 1,2,4-triazole-3-carboxylic acid derivatives, acyloxalamidrazones, antiviral agents, acute lymphoblastic leukemia, chronic myeloid leukemia, antimicrobial properties.

1,2,4-Triazole-3-carboxylic acid is an interesting basic scaffold for various biologically active compounds, including multivalent systems. Among them, the most known are 1,2,4-triazole-3-carboxylic acid nucleoside derivatives which have the greatest structural diversity.[1–4] For example, ribavirin and its derivatives containing 5-substituted 1,2,4-triazole-3-carboxamide moieties as heterocyclic bases exhibit a broad spectrum of biological properties.[5,6] However, nucleosides with a 5-positioned amino group in the 1,2,4-triazole fragment are less represented. Among them, 5-aminoribavirin[7] and its *N*-aryl derivatives are known, the latter demonstrating antitumor activity.[8] This generates interest in the biological properties of other derivatives of 5-amino-1,2,4-triazole-3-carboxylic acid, in particular, to the relative carboxamide **1**.



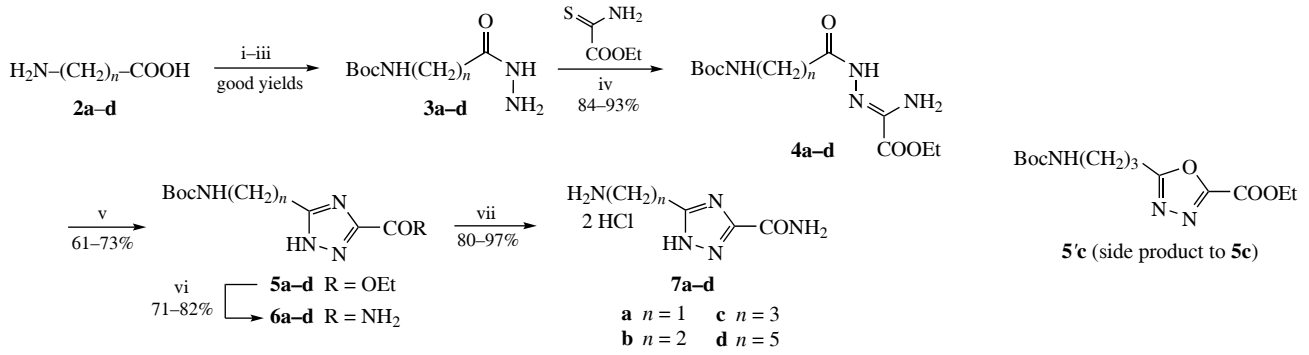
1

In the cases of ribavirin analogues not only nucleosides but also their heterocyclic bases often exhibit biological activity.[9,10] For example, 5-substituted 1,2,4-triazole-3-carboxamide and 1,2,4-triazole-3-carboxylate demonstrated superior activity to that of their ribosides and 2-hydroxyethoxymethyl analogues in a study of activity against RNA containing tobacco mosaic virus.[11–13]

Therefore, we were interested in a number of 5-amino-1,2,4-triazole-3-carboxamide homologues as potential bioactive compounds and in comparing their properties with ribavirin. For this purpose, we needed a method that would allow us to obtain these homologues in preparative quantities. Synthetic routes to 5-amino-1,2,4-triazole-3-carboxamide **1** and its homologues are different,[14,15] therefore, we used two strategies for their preparative synthesis. 5-Amino-1,2,4-triazole-3-carboxamide **1** was obtained from oxalic acid and aminoguanidine bicarbonate in a two-step sequence through intermediate 5-amino-1,2,4-triazole-3-carboxylic acid and its chloride with an overall yield of 59% (see Online Supplementary Materials).

The synthetic access to homologous 5-(ω-aminoalkyl)-1,2,4-triazole-3-carboxylic acids may be in principle close to that for their simpler 5-alkyl analogues. For the latter, the known methods are mainly comedown to the cyclization of acylamidrazones or diacylated amidrazones.[16,17] In this work, we designed the corresponding synthetic sequence starting from ω-amino-alkanoic acids **2a–d** (Scheme 1).

The crucial step in this scheme had to be thermal cyclization of acylamidrazones **4a–d**. The case that involved the cyclization of precursor **4a** affording ethyl 5-[*N*-(*tert*-butoxycarbonyl)-aminomethyl]-1,2,4-triazole-3-carboxylate **5a** was previously documented.[15] We decided to use it as a basis for the preparative synthesis of other homologues **5b–d**. However, this method has some limitations, for example, the pH dependent formation of 1,3,4-oxadiazole heterocycle along with the target 1,2,4-triazole.[18] In this work, we performed selection of the reaction conditions using the example of acylamidrazone **4c** having the medium length of the hydrocarbon linker in the series. Thermal cyclization of **4c** was carried out in the presence of both acidic



Scheme 1 *Reagents and conditions*: i, $SOCl_2$, MeOH (anhydrous), room temperature; ii, Boc_2O, Et_3N, MeOH, room temperature; iii, $N_2H_4 \cdot H_2O$, MeOH, room temperature; iv, EtOH, room temperature; v, *o*-xylene, reflux; vi, NH_3, MeOH, reflux; vii, HCl, dioxane (anhydrous), room temperature.

and basic catalysts, as well as without them. Due to the presence of labile *tert*-butoxycarbonyl and ester groups in amidrazones **4** we tested *p*-toluenesulfonic acid (TsOH) or pyridine as cyclization catalysts. The product composition was assessed by HPLC with mass spectrometric detector using authentic ethyl 5-[3-(*tert*-butyloxycarbonylamino)propyl]-1,2,4-triazole-3-carboxylate **5c** and ethyl 5-[3-(*tert*-butoxycarbonylamino)-propyl]-1,3,4-oxadiazole-2-carboxylate **5′c** as the standards. The highest content of 1,2,4-triazole **5c** was observed in the case of 2-hour refluxing in *o*-xylene without the addition of a catalyst (Table 1). The presence of pyridine resulted in a slight decrease in the content of 1,2,4-triazole **5c** while the presence of TsOH sharply reduced its content. The maximum content of 1,3,4-oxadiazole **5′c** was observed with the application of 0.1 equiv. of TsOH. The increase in TsOH content decreased not only the **5′c** yield but also **4c** conversion after 2 h of reflux. When acyloxalamidrazone hydrochloride **4c** · HCl (obtained by treating acyloxalamidrazone **4c** with two equivalents of 0.2 M HCl in THF) was refluxed in *o*-xylene without the addition of a catalyst, none of the products **5c** and **5′c** were detected even after complete conversion of the starting compound.

Acyloxalamidrazone **4c** was obtained with 67% overall yield in four steps (see Scheme 1): γ-aminobutyric acid **2c** was esterified, and its methyl ester was *N*-protected with a *tert*-butyloxycarbonyl group. Further hydrazinolysis of this derivative gave hydrazide **3c**, which was then reacted with thioxamic acid ethyl ester to form acylamidrazone **4c**. For the preparative synthesis of triazole **5c**, cyclization in the absence of a catalyst was chosen. Under these conditions, the preparative yield of **5c** was 61%, with an increase in the duration of boiling to 12 h and an isolation of **5c** by column chromatography on silica gel in methanol–chloroform eluent.

Thermal catalyst-free cyclization in *o*-xylene was also chosen for the preparative synthesis of other homologues **5a**,**b**,**d** (see Scheme 1) which were obtained in 62–73%. Amides **6a–d** were prepared by ammonolysis of esters **5a–d** and isolated by column chromatography on silica gel with 71–82% yields. The removal of Boc-protection was performed by the action of 3.4 M HCl/1,4-dioxane solution, which afforded the target 5-substituted 1,2,4-triazole-3-carboxamide dihydrochlorides **7a–d** with 80–97% yields. The structures of all obtained compounds were assessed by a set of physicochemical methods of analysis including ^1H, ^{13}C NMR spectroscopy and high resolution mass spectrometry. The purity of the products, determined by the high performance liquid chromatography with mass spectrometric detector, was more than 95%.

Table 1 Yield of the products of the oxalamidrazone **4c** cyclization.[a]

Catalyst	Yield (%)	
	1,2,4-triazole **5c**	1,3,4-oxadiazole **5′c**
Pyridine (50 mol%)	42	2
Pyridine (30 mol%)	43	4
Pyridine (20 mol%)	46	4
Pyridine (10 mol%)	47	6
Pyridine (5 mol%)	51	7
No catalyst	65	8
TsOH (5 mol%)	15	32
TsOH (10 mol%)	10	44
TsOH (20 mol%)	–[b]	28
TsOH (30 mol%)	–[b]	10
TsOH (50 mol%)	–[b]	9

[a] From HPLC-MS data. [b] The product was not detected.

As mentioned above, derivatives of 1,2,4-triazole heterocyclic base can sometimes exhibit biological activity comparable with that of ribavirin nucleoside. Therefore, we studied the spectrum of biological properties of the synthesized series of homologues, namely their antiviral, antitumor and antimicrobial properties, in comparison with ribavirin. The effect against the influenza A/Aichi/2/68 (H3N2) RNA virus was estimated on the MDCK cell model. It was shown that compounds **1** and **7a–d** exhibited lower cytotoxic concentrations (CC_{50}) than ribavirin. As a result of assessing the antiviral properties, it was found that all homologues suppressed virus replication to varying degrees. Compound **7d** at a concentration of 1 mM completely suppressed the development of the A/Aichi/2/68 (H3N2) virus, as ribavirin did (see Online Supplementary Materials). In CCRF-SB acute lymphoblastic leukemia and K562 chronic myeloid leukemia cell lines, compound **7a**, unlike ribavirin, increases the proportion of cells in the G0 phase of the cell cycle, similar to the antitumor drug cytarabine. Weak antimicrobial effect of **1** and **7b–d** was demonstrated against *M. luteus*, *S. aureus*, *P. aeruginosa*, while ribavirin demonstrated weak activity against *P. aeruginosa* and *C. albicans*. Antimicrobial activity was also assessed on a non-pathogenic model of *M. tuberculosis–M. smegmatis*. Extremely low inhibition of microorganism growth was revealed in the presence of synthesized compounds **7b–d**.

To summarize, using the catalyst-free thermal cyclization of *N*-acylated amidrazones, new homologues of 5-amino-1,2,4-triazole-3-carboxamide were obtained in preparative quantities, and the spectrum of their biological action was assessed. Using a cell model of the influenza A/Aichi/2/68 (H3N2) RNA virus, it was shown that compound **7d** exhibited an antiviral effect comparable to that of ribavirin at concentrations of 1 mM with half the toxicity. The toxicity of homologues **7a–d** against acute and chronic forms of leukemia is inferior to the toxicity of ribavirin, but analogue **7a** increases the proportion of cells in the G0 phase of the cell cycle, similar to the antitumor drug cytarabine. The resulting series of homologues demonstrates an antimicrobial profile different from that of ribavirin. Synthesized homologues **7b–d** exhibit low effect against both gram-positive and gram-negative bacteria, unlike ribavirin, which exhibits

activity only against the gram-negative bacteria *P. aeruginosa* and *C. albicans*. Therefore, homologues of 5-amino-1,2,4-triazole-3-carboxamide are promising not only as heterocyclic bases for synthetic nucleosides, but may become the basis for the development of a new class of non-nucleoside biologically active agents.

Anticancer effects explorer was supported by the Russian Science Foundation (RSF) grant no. 23-25-00382. The work of O. N. Sineva was carried out as part of institutional funding of the Gause Institute of New Antibiotics. Current work in its synthetic part was performed using the equipment of the Shared Science and Training Center for Collective Use RTU MIREA and supported by the Ministry of Science and Higher Education of the Russian Federation.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.71267/mencom.7753.

References

1 Y. Fan, Y. Xia, J. Tang, P. Rocchi, F. Qu, J. Iovanna and L. Peng, *Org. Lett.*, 2010, **12**, 5712; https://doi.org/10.1021/ol102537p.
2 M. McDowell, S. R. Gonzales, S. C. Kumarapperuma, M. Jeselnik, J. B. Arterburn and K. A. Hanley, *Antiviral Res*., 2010, **87**, 78; https://doi.org/10.1016/j.antiviral.2010.04.007.
3 N. I. Zhurilo, M. V. Chudinov, A. V. Matveev, O. S. Smirnova, I. D. Konstantinova, A. I. Miroshnikov, A. N. Prutkov, L. E. Grebenkina, N. V. Pulkova and V. I. Shvets, *Bioorg. Med. Chem. Lett.*, 2018, **28**, 11; https://doi.org/10.1016/j.bmcl.2017.11.029.
4 Q. Guan, S. Xing, L. Wang, J. Zhu, C. Guo, C. Xu, Q. Zhao, Y. Wu, Y. Chen and H. Sun, *J. Med. Chem*., 2024, **67**, 7788; https://doi.org/10.1021/acs.jmedchem.4c00652.
5 Y. Xia, M. Wang, E. Beraldi, M. Cong, A. Zoubeidi, M. Gleave and L. Peng, *Anticancer Agents Med. Chem*., 2015, **15**, 1333; https://doi.org/10.2174/1871520615666150617110943.
6 Y. Xia, F. Qu and L. Peng, *Mini-Rev. Med. Chem.,* 2010, **10**, 806; https://doi.org/10.2174/138955710791608316.
7 S. R. Naik, J. T. Witkowski and R. K. Robins, *J. Heterocycl. Chem.*, 1974, **11**, 57; https://doi.org/10.1002/jhet.5570110112.
8 Y. Liu, Y. Xia, Y. Fan, A. Maggiani, P. Rocchi, F. Qu and L. Peng, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 2503; https://doi.org/10.1016/j.bmcl.2010.02.104.
9 E. A. Mikhina, D. V. Stepanycheva, V. P. Maksimova, O. N. Sineva, N. N. Markelova, L. E. Grebenkina, E. A. Lesovaya, M. G. Yakubovskaya, A. V. Matveev and E. M. Zhidkova, *Molecules*, 2024, **29**, 4808; https://doi.org/10.3390/molecules29204808.
10 M. V. Chudinov, *Fine Chem. Technol.*, 2019, **14**, 7; https://doi.org/10.32362/2410-6593-2019-14-4-7-23.
11 Y. Xia, Z. Fan, J. Yao, Q. Liao, W. Li, F. Qu and L. Peng, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 2693; https://doi.org/10.1016/j.bmcl.2006.02.023.
12 M. Wang, R. Zhu, Z. Fan, Y. Fu, L. Feng, J. Yao, A. Maggiani, Y. Xia, F. Qu and L. Peng, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 354; https://doi.org/10.1016/j.bmcl.2010.10.141.
13 Y. Xia, W. Li, F. Qu, Z. Fan, X. Liu, C. Berro, E. Rauzy and L. Peng, *Org. Biomol. Chem.*, 2007, **5**, 1695; https://doi.org/10.1039/b703420b.
14 L. Kukuljan and K. Kranjc, *Tetrahedron Lett.*, 2018, **60**, 207; https://doi.org/10.1016/j.tetlet.2018.12.014.
15 S. Borg, G. Estenne-Bouhtou, K. Luthman, I. Csoeregh, W. Hesselink and U. Hacksell, *J. Org. Chem*., 1995, **60**, 3112; https://doi.org/10.1021/jo00115a029.
16 J. E. Francis, L. A. Gorczyca, G. C. Mazzenga and H. Meckler, *Tetrahedron Lett*., 1987, **28**, 5133; https://doi.org/10.1016/s0040-4039(00)95610-7.
17 M. V. Chudinov, A. V. Matveev, N. I. Zhurilo, A. N. Prutkov and V. I. Shvets, *J. Heterocycl. Chem.*, 2014, **52**, 1273; https://doi.org/10.1002/jhet.1934.
18 J. Dost, J. Stein and M. Heschel, *Z. Chem*., 2010, **26**, 203; https://doi.org/10.1002/zfch.19860260603.

Received: 26th February 2025; Com. 25/7753