Mendeleev Communications

Mendeleev Commun., 2025, **35**, 433–436

Derivatives of sterically hindered phenols and pyridinecarboxylic acids as prospective radioprotectors

Evgeny A. Nikitin,*[a] Zahra A. Ahmedova,[a] Maria A. Kolyvanova,[a,b,c] Nikita S. Lifanovsky,[b] Alexandr V. Belousov,[c] Alina A. Markova,[b] Minh Tuan Nguyen,[b] Elena F. Shevtsova,[d] Konstantin A. Lyssenko,[a] Vladimir Yu. Tyurin,[a] Vladimir A. Kuzmin,[b] Vladimir N. Morozov*[b] and Elena R. Milaeva[a,d]

[a] *Department of Chemistry, M. V. Lomonosov Moscow State University, 119991 Moscow, Russian Federation. E-mail: evgeny.a.nikitin@yandex.ru*

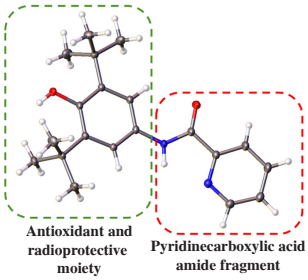
[b] *N. M. Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, 119334 Moscow, Russian Federation. E-mail: morozov.v.n@mail.ru*

[c] *Burnasyan Federal Medical Biophysical Center, Federal Medical Biological Agency of the Russian Federation, 123098 Moscow, Russian Federation*

[d] *Institute of Physiologically Active Compounds, Federal Research Center of Problems of Chemical Physics and Medicinal Chemistry, Russian Academy of Sciences, 142432 Chernogolovka, Moscow Region, Russian Federation*

DOI: 10.71267/mencom.7671

New compounds consisting of 2,6-di-*tert*-butylphenol and isomeric pyridinecarboxamide moieties as well as their water-soluble hydrochlorides were synthesized. The compounds appeared to be potent antioxidants and radioprotectors, with no significant difference in activity between the pyridine bases and their salts. The cytotoxicity assay showed no significant toxic impact from any of the substances, making them promising candidates for further investigation.



Keywords: radioprotectors, antioxidants, radical scavengers, sterically hindered phenols, pyridinecarboxamides, circular dichroism.

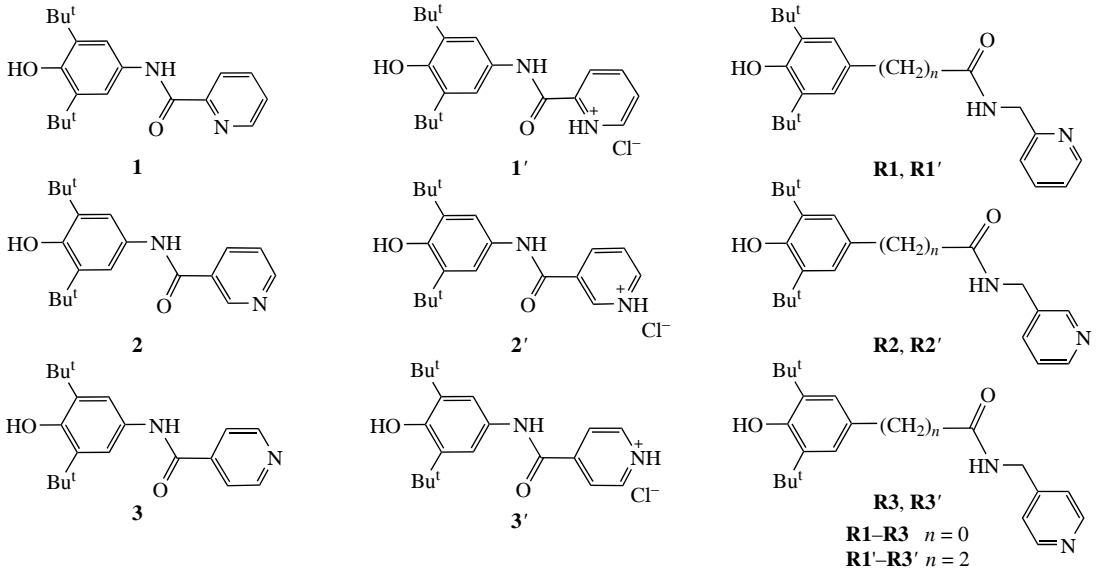
In most cases, damage in living systems induced by ionizing radiation *in situ* is mediated by reactive oxygen species, *e.g.* OH^-, $HO^•$, $O_2^{-•}$, H_2O_2.[1] Their action is called indirect and contribute about 80–90% to the biological effect, while the remaining is related to its direct action.[2] In contrast to the latter, radical processes may be suppressed using specialized free radical scavengers defined as radioprotectors.[3] Nowadays, the main task in the development of chemical protection against radiation injuries remains the search for new compounds that combine both high efficacy and low toxicity. Considerable attention in this issue is paid to natural and synthetic antioxidants (α-tocopherol, melatonin, glutathione, *etc.*).[4–6] Among others, strong radical-scavenging properties were also found in sterically hindered phenols. Ionol (2,5-di-*tert*-butyl-4-methylphenol, BHT), the most known representative of this family, is used as a liniment, which has a regenerative and wound-healing effect and exhibits moderate antioxidant activity (Table 1). Their another feature is the possibility of chemical modification and ease of synthesis, which allows the use of hindered phenol scaffold as a convenient platform for the creation of new compounds (indeed, most commercial synthetic hindered phenolic antioxidants are based upon BHT).[7] Moreover, modification of such structures in the *para* position by fragments with own biological activity can significantly expand their functionality.[8] It is, however, not always the case that the antioxidant properties of a compound are enhanced. Structurally related derivatives **R1**–**R3**/**R1′**–**R3′** consisting of 2,6-di-*tert*-butylphenol and picolylcarbamoyl units[9] exhibited mild antioxidant activity over a prolonged period (see Table 1), demonstrating a dependence of protective properties on the linker length. These molecules, however, did not contain any natural antioxidant groups in their structure.

Pyridinecarboxylic acids, on the other hand, exhibit antioxidant properties in model tests only at high concentrations.[10] Their *in vivo* efficacy is due to their low toxicity and complex mechanism of action, which involves, for example, cellular membrane stabilization. Despite a significant amount of works on the antioxidant properties of sterically hindered phenols,[11–14] very few compounds have been studied in

Table 1 Antioxidant activity of compounds **1**–**3** and their hydrochlorides **1′**–**3′** measured in DPPH (EC_{50}), CUPRAC (TEAC), LOX inhibition (I) tests, as well as the IC_{50} values for LP inhibition.

Compound	EC_{50}/mM	TEAC	I (%)	IC_{50}/mM
1	0.10±0.03	0.95±0.08	42.9±4.1	1.45±0.15
2	0.04±0.02	0.68±0.06	44.0±4.8	1.63±0.19
3	0.06±0.02	0.78±0.07	50.6±5.5	1.83±0.29
1′	0.06±0.02	0.67±0.06	37.0±3.8	1.35±0.10
2′	0.16±0.03	0.80±0.07	10.5±2.9	1.42±0.24
3′	0.20±0.04	1.05±0.09	16.8±3.0	1.47±0.30
BHT (Tonarol)	0.10±0.01	1.10±0.03	50.0±1.3	1.40±0.10
R1	n/a[a]	0.89±0.06	7.6±2.1	22.8±6.4
R2	n/a	0.29±0.03	8.5±3.5	14.0±2.8
R3	n/a	0.35±0.03	12.0±4.5	24.1±6.4
R1′	n/a	1.71±0.09	1.4±0.3	3.4±0.8
R2′	n/a	1.00±0.07	5.3±1.8	3.4±0.7
R3′	n/a	1.10±0.08	2.6±0.6	3.9±1.0

[a] n/a – not active.



combination with ionizing radiation. This appears to be a serious omission since the radioprotection efficacy cannot be unambiguously assessed based solely on antioxidant activity.

In this work, we present a series of new radioprotectors based on sterically hindered phenol core, substituted in the *para* position with the residue of nicotinic acid,[15] known for its own antioxidant properties, as well as its isomers (compounds **1**–**3**). These compounds were accessed routinely by the acylation of 4-amino-2,6-di-*tert*-butylphenol with the isomeric pyridinecarboxylic acids using EDC·HCl/DMAP system[16] (see also Online Supplementary Materials, Scheme S1). Since compounds **1**–**3** are soluble only in organic solvents, their water-soluble hydrochlorides **1′**–**3′** were obtained by treatment with concentrated HCl in MeOH at 70 °C.[17] Their characterization was performed *via* IR, ^{1}H and ^{13}C NMR spectroscopy, as well as elemental and X-ray diffraction analysis of amide **1** (Figure 1).†

Antioxidant properties of the synthetized compounds were estimated in an array of model assays, the results of which are summarized in Table 1 (herewith, in all cases, BHT was used as the standard) and in Figure 2. Their electron-donating properties were estimated in the test with stable chromophore radical 2,2-diphenyl-1-picrylhydrazyl (DPPH).[18] The measured activity values are reported as EC_{50}. The capacity of **1**–**3** and their hydrochlorides **1′**–**3′** to abstract single electron was also estimated colorimetrically *via* reducing cupric antioxidant capacity (CUPRAC-test; reduction of copper(II) to copper(I) in its complex with two molecules of neocuproine is accompanied by a change of the solution's color from light blue to yellow-orange).[19] The corresponding results are expressed in Trolox equivalent antioxidant capacity (TEAC). The abilities of the synthetized compounds to prevent lipid peroxidation (LP), as well as to inhibit lipoxygenase (LOX) enzyme and to halt direct Fe^{2+}-induced LP were investigated in addition. The LOX inhibition capacity was tested on soybean lipoxygenase (LOX 1-B) according to known procedure[17] and expressed as inhibition percentage *I*. In turn, LP was carried out on rat liver homogenate according to existing procedure.[20] The values of IC_{50} were calculated from corresponding concentration dependences of LP activity (see Figure 2). It is clearly seen that, even though the relative activity of the studied compounds varies markedly in different tests, all of them demonstrate pronounced antioxidant

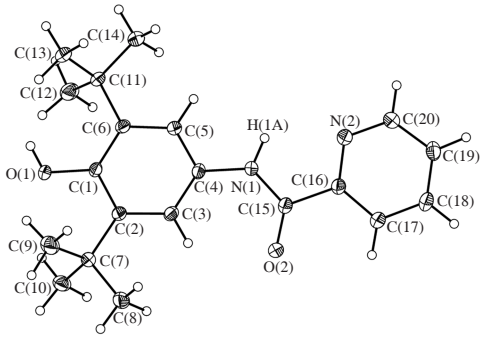


Figure 1 The general view of compound **1** in representation of atoms by thermal ellipsoids (*p* = 50%).

† *Crystal data for* **1**. Reddish crystals suitable for X-ray analysis were obtained by slow crystallization from the CH_2Cl_2–light petroleum mixture (1 : 1) under normal conditions for one day. $C_{20}H_{26}N_2O_2$ (*M* = 326.43), monoclinic, space group $P2_1/c$ at 105 K, *a* = 9.5288(4), *b* = 16.3497(7) and *c* = 11.9012(4) Å, *β* = 107.777(2)°, *V* = 1765.59(12) Å^3, *Z* = 4, (*Z′* = 1), d_{calc} = 1.228 g cm^{-3}. Total of 17171 reflections were collected (4697 independent reflections, θ_{max} = 29°, R_{int} = 0.0523). The refinement converged to R_1 = 0.0510 (for 3924 observed reflections), GOF = 1.044.

CCDC 2388353 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* https://www.ccdc.cam.ac.uk.

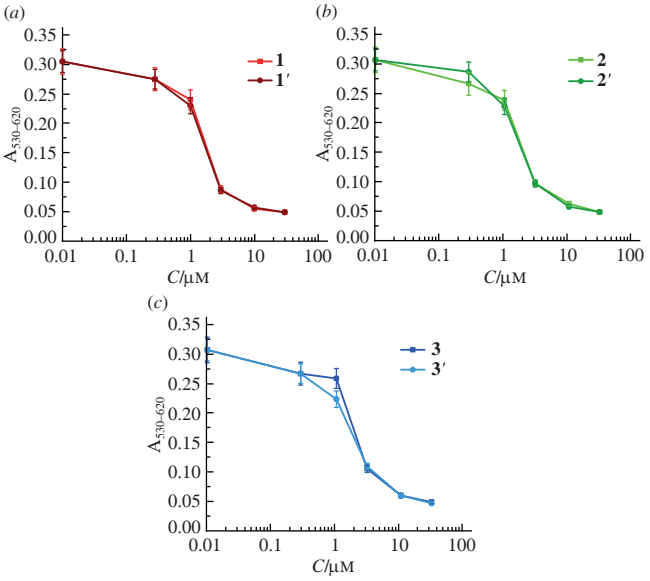


Figure 2 Concentration dependences of LP activity for the compounds **1**–**3** and their hydrochlorides **1′**–**3′**.

properties comparable to those of BHT and Trolox. For example, in CUPRAC-test, **2′** and **3′** are more active than **2** and **3**, while in LOX inhibition test the pattern is reverse. Nevertheless, as the overall conclusion to the performed model assays, one can point that there is no vast differences in antioxidant activity between the pyridine bases **1**–**3** and their salts **1′**–**3′**. This means that both forms can be used with almost equal efficacy, depending on whether lipophilic or hydrophilic properties are crucial in a concrete case. The properties of the new compounds may be compared with those for their structural analogs **R1**–**R3**/**R1′**–**R3′** (*cf.* ref. 9).

Salts **1′**–**3′** were then tested to protect DNA molecules from radiation damage. To do this, we used the ability of DNA molecules of low molecular weight [(0.25–0.5)×10^6 Da, *i.e.*, 400–800 base pairs] to form optically active dispersed mesophases (also known as cholesteric liquid-crystalline dispersions) under the phase exclusion conditions (for details, see ref. 21). Since the amplitude of the characteristic circular dichroism (CD) signal of such a system is directly related to the integrity of DNA molecules, the degree of protection (the radioprotector efficacy) can be assessed by its relative change.[22] Figure 3(*a*),(*b*), shows the CD spectra of the systems assembled from DNA molecules irradiated with X-rays (for details, see ref. 23) in the absence and in the presence of **3′** (10^{-4} M), respectively. It is clearly seen that the addition of this compound before irradiation notably reduces the level of DNA damage over the entire range of studied doses. For example, almost complete protection is observed at 250 Gy, and at 1000 Gy in the presence of **3′** the CD signal is almost 2.9-fold more intense. Figure 3(*c*) summarizes the experimental results for the entire line of compounds studied. Although statistically significant radioprotective effect is found for all of them, it is the salt **3′** that demonstrates the highest efficacy (**2′** is in second place, while **1′** has the weakest effect).

Since compounds **1′**–**3′** are expected to act in biological environments, it is crucial to evaluate their toxic effects on living cells. The survival of human cells was studied *in vitro* on the example of colon adenocarcinoma (HCT116) and non-tumor fibroblast (WI38) cell lines *via* MTT-test. Compounds **1′**–**3′** were incubated for 72 h in the concentration range up to 50 μM (doxorubicin was used as control). The obtained survival curves

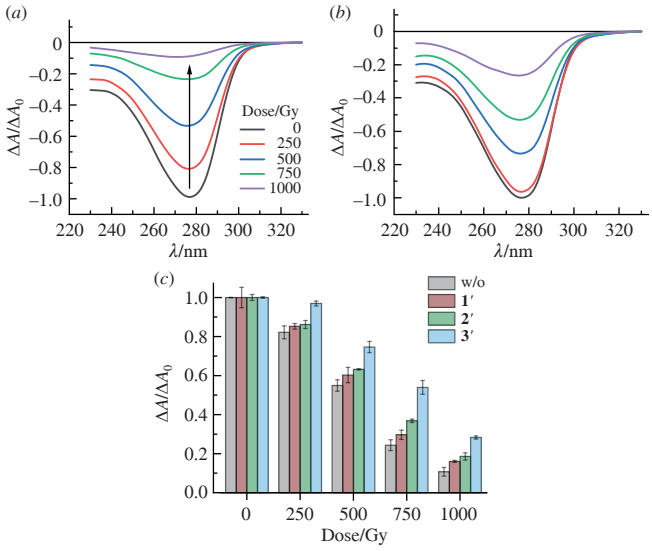


Figure 3 CD spectra of dispersed mesophases prepared from DNA molecules preliminarily irradiated with 0–1000 Gy (*a*) in the absence and (*b*) in the presence of 10^{-4} M **3′**. (*c*) Relative radioprotective efficacy of compounds **1′**–**3′** compared to the non-threated control (in all cases the concentration was 10^{-4} M). Error bars indicate the standard deviations calculated from 3 independent measurements.

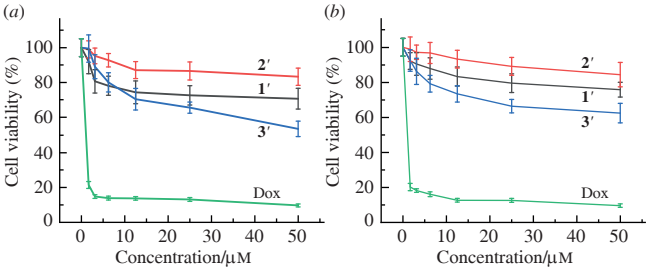


Figure 4 Survival curves for (*a*) HCT116 and (*b*) WI38 cell lines. Error bars indicate the standard deviations calculated from three independent measurements.

are shown in Figure 4. It is clearly seen that the studied compounds are characterized by rather low cytotoxicity towards both tumor and healthy cells: within the studied concentration range, it is not possible to determine the LD_{50} values for both cases. However, the salts still differ from each other in the magnitude of their cytotoxic effect (according to this parameter they are ranked as follows: **3′** > **1′** > **2′**; note that for free bases **1**–**3** the effect was completely similar). This correlates well with the well-known rule of thumb for radioprotectors (this principle, of course, is not absolute): the more effective the compound, the higher its toxicity.

In conclusion, the synthesized 2,6-di-*tert*-butylphenol-pyridinecarboxamide hybrids demonstrate pronounced antioxidant activity in model assays *in vitro* as well as radioprotective properties in tests utilizing ionizing radiation. Considering their low toxicity, we can propose them as promising candidates in novel radioprotectors.

This work was supported by The Russian Science Foundation (grant no. 23-23-10030). Cell cultivation and MTT-assay were performed in the Core Facility of the Emanuel Institute of Biochemical Physics of Russian Academy of Sciences ‘New materials and technologies’. Authors acknowledge support from Lomonosov Moscow State University Program of Development for providing access to the single X-ray diffraction facilities.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.71267/mencom.7671.

References

1 O. Desouky, N. Ding and G. Zhou, *J. Radiat. Res. Appl. Sci.*, 2015, **8**, 247; https://doi.org/10.1016/j.jrras.2015.03.003.
2 Y. N. Korystov, *Radiat. Res.*, 1992, **129**, 228; https://doi.org/10.2307/3578162.
3 L. M. Rozhdestvensky, *Radiat. Biol., Radioekol.*, 2017, **57**, 117 (in Russian); https://doi.org/10.7868/S0869803117020126.
4 J. F. Weiss and M. R. Landauer, *Ann. N. Y. Acad. Sci.*, 2000, **899**, 44; https://doi.org/10.1111/j.1749-6632.2000.tb06175.x.
5 C. K. Nair, D. K. Parida and T. Nomura, *J. Radiat. Res.*, 2001, **42**, 21; https://doi.org/10.1269/jrr.42.21.
6 S. V. Gudkov, N. R. Popova and V. I. Bruskov, *Biophysics*, 2015, **60**, 659; https://doi.org/10.1134/S0006350915040120.
7 K. D. Breese, J.-F. Lamèthe and C. DeArmitt, *Polym. Degrad. Stab.*, 2000, **70**, 89; https://doi.org/10.1016/S0141-3910(00)00094-X.
8 E. R. Milaeva, *Curr. Top. Med. Chem.*, 2011, **11**, 2703; https://doi.org/10.2174/156802611798040741.
9 E. A. Nikitin, D. B. Shpakovsky, A. D. Pryakhin, T. A. Antonenko, V. Yu. Tyurin, A. A. Kazak, A. N. Ulyanov, V. A. Tafeenko, L. A. Aslanov, L. G. Dubova, E. A. Lysova, E. F. Shevtsova and E. R. Milaeva, *Pharm. Pharmacol. Int. J.*, 2020, **8**, 122; https://doi.org/10.15406/ppij.2020.08.00288.
10 R. K. Ameta and M. Singh, *J. Mol. Liq.*, 2014, **195**, 40; https://doi.org/10.1016/j.molliq.2014.01.029.
11 N. M. Storozhok, N. V. Gureeva, A. P. Krysin, V. E. Borisenko, I. F. Rusina, N. G. Khrapova and E. B. Burlakova, *Kinet. Catal.*, 2004, **45**, 488; https://doi.org/10.1023/B:KICA.0000038075.46269.a6.

12 D. V. Aref'ev, I. S. Belostotskaya, V. B. Vol'eva, N. S. Domnina, N. L. Komissarova, O. Yu. Sergeeva and R. S. Khrustaleva, *Russ. Chem. Bull.*, 2007, **56**, 781; https://doi.org/10.1007/s11172-007-0117-x.
13 M. N. Kolyada, V. P. Osipova, N. T Berberova, D. B. Shpakovsky and E. R. Milaeva, *Russ. J. Gen. Chem.*, 2018, **88**, 2513; https://doi.org/10.1134/S1070363218120095.
14 A. A. Antonets, K. M. Voroshilkina, I. A. Shutkov, D. M. Mazur, V. Yu. Tyurin, L. G. Dubova, E. F. Shevtsova, A. A. Nazarov and E. R. Milaeva, *Mendeleev Commun.,* 2024, **34**, 74; https://doi.org/10.1016/j.mencom.2024.01.022.
15 J. P. Kamat and T. P. Devasagayam, *Redox Rep.*, 1999, **4**, 179; https://doi.org/10.1179/135100099101534882.
16 M. Tsakos, E. S. Schaffert, L. L. Clement, N. L. Villadsen and T. B. Poulsen, *Nat. Prod. Rep.*, 2015, **32**, 605; https://doi.org/10.1039/C4NP00106K.
17 E. Nikitin, E. Mironova, D. Shpakovsky, Y. Gracheva, D. Koshelev, V. Utochnikova, K. Lyssenko, Y. Oprunenko, D. Yakovlev, R. Litvinov, M. Seryogina, A. Spasov and E. Milaeva, *Molecules*, 2022, **27**, 8359; https://doi.org/10.3390/molecules27238359.
18 W. Brand-Williams, M. E. Cuvelier and C. Berset, *LWT – Food Sci. Technol.*, 1995, **28**, 25; https://doi.org/10.1016/S0023-6438(95)80008-5.
19 R. Apak, K. Güçlü, M. Ozyürek and S. E. Karademir, *J. Agric. Food Chem.*, 2004, **52**, 7970; https://doi.org/ 10.1021/jf048741x.
20 W. L. Erdahl, R. J. Krebsbach and D. R. Pfeiffer, *Arch. Biochem. Biophys.*, 1991, **285**, 252; https://doi.org/ 10.1016/0003-9861(91)90357-o.
21 Yu. M. Yevdokimov and V. V. Sytchev, *Russ. Chem. Rev.*, 2008, **77**, 193; https://doi.org/10.1070/RC2008v077n02ABEH003734.
22 M. A. Kolyvanova, N. S. Lifanovsky, E. A. Nikitin, M. A. Klimovich, A. V. Belousov, V. Y. Tyurin, V. A. Kuzmin and V. N. Morozov, *High Energy Chem.*, 2024, **58**, 134; https://doi.org/10.1134/S0018143924010107.
23 M. A. Kolyvanova, M. A. Klimovich, A. V. Belousov, V. A. Kuzmin and V. N. Morozov, *Photonics*, 2022, **9**, 787; https://doi.org/10.3390/photonics9110787.

Received: 29th October 2024; Com. 24/7671