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Study of 1-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2-arylbenzimidazoles Antioxidant Activity

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Abstract—Antioxidant activity of benzimidazole derivatives with sterically hindered phenol substituent has been estimated using the modified procedure. The compounds antioxidant activity is strongly correlated with the potential of peroxide oxidation of the corn oil lipids of polyunsaturated aliphatic acids.

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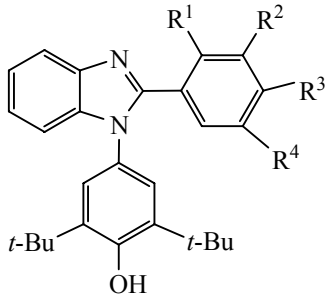
The studies of compounds containing fragments of sterically hindered (shielded) phenols are of priority importance; due to special features of the structure and chemical behavior of such compounds, they are used in a wide range of practical applications, including healthcare industry [1, 2].

Being efficient inhibitors of the free radical processes, the compounds of this class are used to protect various organic materials from oxidative and thermal destructions. Moreover, many of these compounds are highly biologically active, and efficient antioxidants and antihypoxic and neuroprotective drugs are found among them [3, 4].

The study of correlations of the antioxidant biological activity and the redox activity is an important part of efficient antioxidant drugs development. A promising approach is to combine the hindered phenol group and the active functional fragment (for instance, heterocyclic one) in the same molecule. Recently, it has been demonstrated that the compounds containing both benzimidazole fragment and a hindered phenol group show a set of excellent utilitarian properties, including antioxidant activity [5].

The *in vitro* tests of synthetic antioxidants in the specially designed systems is a simple procedure to estimate the antioxidant activity judging from the ability to inhibit the free radical processes; the radicals in the testing system may appear via chemical or physical initiation.

Previously, 1-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2-arylbenzimidazoles **I–XII** were prepared, and their redox activity was studied [6]. Extending that study, in this work we determined the antioxidant activity of the same set of compounds taking advantage of a modified testing procedure. The testing system contained a mixture of saturated and unsaturated aliphatic acids from corn oil, the peroxide oxidation was induced either physically (UV irradiation) or chemically (Fenton's reagent, Fe^{2+}–H_2O_2) [7]. Butylated hydroxytoluene (ionol, dibunol) and ubiquinone (coenzyme Q_{10}) were used as standard compounds. The activity was expressed in universal (ubiquinone) units.



I–XII

R^1 = OH, R^2 = CH_3, R^3, R^4 = H (**I**); R^1 = OH, R^2 = H, R^3 = CH_3, R^4 = H (**II**); R^1 = OH, R^2, R^3 = H, R^4 = CH_3 (**III**); R^1 = OH, R^2, R^4 = Br, R^3 = H (**IV**); R^1 = OH, R^2, R^3, R^4 = H (**V**); R^1 = H, R^2, R^4 = *t*-Bu, R^3 = OH (**VI**); R^1, R^2, R^3, R^4 = H (**VII**); R^1, R^2, R^4 = H, R^3 = NO_2 (**VIII**); R^1, R^2, R^4 = H, R^3 = Br (**IX**); R^1 = Cl, R^2, R^3, R^4 = H (**X**); R^1, R^2, R^4 = H, R^3 = Cl (**XI**); R^1, R^2, R^4 = H, R^3 = CH_3 (**XII**).

At the first stage of the study the compounds of interest were applied to the testing system at varied concentration (0.25×10^{-5}, 0.5×10^{-5}, 1.0×10^{-5}, and $2.0\times$

Table 1. Antioxidant activity of **I–XII** and the reference compounds in the UV–Fe^{2+} test system (M±*m*; *n* = 5)

Comp. no.	*c*, mol l^{-1}	Absorbance, rel. units		AOA, % (Q-un.)
		λ = 450 nm	λ = 532 nm	
I	1.0×10^{-3}	0.276±0.003	0.074±0.001	65.0 (5.9)
II	1.0×10^{-3}	0.284±0.003	0.116±0.001	60.0 (5.4)
III	1.0×10^{-3}	0.282±0.002	0.108±0.002	61.0 (5.5)
IV	1.0×10^{-3}	0.278±0.003	0.082±0.001	64.0 (5.8)
V	1.0×10^{-3}	0.0274±0.004	0.056±0.001	67.0 (6.1)
VI	1.0×10^{-3}	0.287±0.002	0.133±0.001	58.0 (5.3)
VII	1.0×10^{-3}	0.425±0.01	0.345±0.01	23.0 (2.1)
VIII	1.0×10^{-3}	0.462±0.01	0.428±0.01	11.0 (1.0)
IX	1.0×10^{-3}	0.422±0.004	0.328±0.003	25.0 (2.3)
X	1.0×10^{-3}	0.415±0.01	0.305±0.002	28.0 (2.5)
XI	1.0×10^{-3}	0.404±0.01	0.296±0.004	30.0 (2.7)
XII	1.0×10^{-3}	0.412±0.01	0.298±0.005	29.0 (2.6)
Ubiquinone	1.0×10^{-3}	0.460±0.003	0.430±0.001	11.0 (1.0)
Hydroxytoluene, butylated	1.0×10^{-3}	0.391±0.005	0.219±0.002	39.0 (3.5)

10^{-5} mol l^{-1}). The second stage aimed at determination of the standard compound, butylated hydroxytoluene, antioxidant activity in the testing systems with lipids peroxide oxidation being induced by lower (1.0×10^{-4} mol l^{-1}) and higher (1.0×10^{-3} mol l^{-1}) concentration of Fe(II). According to the results, the antioxidant activity was more prominent in the presence of 1.0×10^{-3} mol l^{-1} of Fe(II) initiator. Finally, the antioxidant activity of all the compounds of interest was determined in the testing systems with peroxide oxidation induced physically (UV–Fe^{2+}) and chemically (Fe^{2+}–H_2O_2). In the preliminary experiments, the optimal biological concentration of 1.0×10^{-3} mol l^{-1} was determined for the both testing systems.

The degree of inhibition was calculated as follows.

$$\%ING = 100 - (I_0/I_K\times100),$$

In that equation, I_0 was the experimental absorbance (450 nm + 532 nm), rel. units; I_K was the reference absorbance (450 nm +5 32 nm), rel. units.

The antioxidant activity AOA was alternatively expressed in the ubiquinone units (Q-un.):

$$AOA\ (Q\text{-un.}) = \%ING_i - \%ING_Q,$$

$\%ING_i$ being the decrease of formation of the products reacting with thiobarbituric acid in the presence of tested compound, $\%ING_Q$ being the decrease of formation of the products reacting with thiobarbituric acid in the presence of ubiquinone.

The ubiquinone antioxidant activity in both the testing systems was taken equal to 100% (or 1.0 Q-un.) as a standard. In the UV–Fe^{2+} system, ubiquinone inhibited lipids peroxide oxidation by 11% (1.0), whereas the inhibition by butylated hydroxytoluene was of 39% (3.5). The highest inhibiting effect in the system with UV initiation was revealed in the cases of **I–V** containing hydroxyl group in the *ortho* position with respect to the hindered phenol fragment conjugated with benzimidazole. The inhibition of free radical oxidation by 1-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2-arylbenzimidazole derivatives **I–VI** was of 58–67%, the antioxidant activity being higher than that of the reference compounds (Table 1). The antioxidant activity of **VIII** was comparable to that of ubiquinone. Other tested compounds showed variable antioxidant effect, slightly higher than that of ubiquinone.

In the testing system with chemical initiation of free radical processes (Fe^{2+}–H_2O_2) the highest inhibiting effect, 56–64%, was achieved in the presence of compounds **I–VI** as well (Table 2). Overall, the antioxidant activity in the latter testing system was lower, thus the tested compounds were less resistant to the chemical initiator of the free radical processes.

Table 2. Antioxidant activity of **I–XII** and the reference compounds in the Fe^{2+}–H_2O_2 test system (M±*m*; *n* = 5)

Comp. no.	*c*, mol l^{-1}	Absorbance, rel. units		AOA, % (Q-un.)
		λ = 450 nm	λ = 532 nm	
I	1.0×10^{-3}	0.278±0.003	0.082±0.001	64.0 (4.0)
II	1.0×10^{-3}	0.323±0.003	0.107±0.001	57.0 (3.6)
III	1.0×10^{-3}	0.321±0.002	0.099±0.002	58.0 (3.6)
IV	1.0×10^{-3}	0.281±0.003	0.109±0.001	61.0 (3.8)
V	1.0×10^{-3}	0.280±0.004	0.090±0.001	63.0 (3.9)
VI	1.0×10^{-3}	0.326±0.003	0.114±0.001	56.0 (3.5)
VII	1.0×10^{-3}	0.432±0.01	0.368±0.01	20.0 (1.2)
VIII	1.0×10^{-3}	0.474±0.01	0.446±0.01	8.0 (0.5)
IX	1.0×10^{-3}	0.430±0.004	0.360±0.003	21.0 (1.3)
X	1.0×10^{-3}	0.422±0.01	0.358±0.002	22.0 (1.4)
XI	1.0×10^{-3}	0.419±0.006	0.311±0.004	27.0 (1.7)
XII	1.0×10^{-3}	0.429±0.006	0.351±0.005	22.0 (1.4)
Ubiquinone	1.0×10^{-3}	0.450±0.003	0.390±0.001	16.0 (1.0)
Hydroxytoluene, butylated	1.0×10^{-3}	0.398±0.005	0.237±0.002	36.5 (2.3)

We tested the correlation of **I–XII** antioxidant activity determined in this work and the oxidative potential of the same compounds reported in [6]. For both of the testing systems, strong correlation was revealed (r^2 = 0.993). That the antioxidant activity towards variously induced free radical processes was in line with the oxidation potential, allowed for reasonably accurate prediction of the antioxidant activity basing on the studies of redox properties.

EXPERIMENTAL

The absorbance was measured with SF-46 spectrophotometer. Statistical data analysis was performed using Statistica 6.0 (StatSoft) and Excel 2007 software packages taking advantage of the paired Student test (*t*). Correlation of the parameters was estimated from *r* coefficient (Pearson method) [8].

Determination of 1-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2-arylbenzimidazoles *in vitro* antioxidant activity with single peroxide oxidation induction by Fenton's system H_2O_2–Fe^{2+}. The aqueous alcohol mixture with oil was prepared as follows: 800 μl of corn oil was added to 3 ml of alcohol and was shaken till emulsification, then the mixture was diluted with distilled water in 100 ml flask upon stirring and shaking. In the centrifuge tube, the reaction mixture was then prepared (2 ml of the oil emulsion, 100 μl of the 10% solution of tested compound, 200 μl of 10% $FeSO_4$ solution, and 10 μl of 3% H_2O_2 solution) and incubated at 37°C during 60 min. The reference specimen was prepared similarly, using 100 μl of distilled water instead of the tested compound solution. After the incubation, 1 ml of 28% trichloroacetic acid solution was added, and the mixture was centrifuged during 10 min at 6000 rpm. 2 ml of supernatant was transferred into a large tube, 1 ml of 1% thiobarbituric acid solution was added, and the resulting sample was kept in a boiling water bath during 15 minutes. The solution absorbance was measured at 450 and 532 nm, with 450 nm corresponding to the absorption maximum of the peroxide oxidation intermediates (diene conjugates) and 532 nm corresponding to the absorbance maximum of the final oxidation product, malonic dialdehyde.

For comparison, the antioxidant activity of butylated hydroxytoluene (dibunol) and ubiquinone was determined. To the above-described testing system 100 μl of the corresponding tested solution was added (in the case of ubiquinone, the tested solution was prepared by dissolution of 1 coenzyme Q_{10} capsule in 10 ml of distilled water), and treated as described above.

Determination of 1-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2-arylbenzimidazoles *in vitro* antioxidant activity with prolonged peroxide oxidation induc-

tion by UV irradiation. The tested compound solution and aqueous alcohol mixture with oil were prepared as described above. In the centrifuge tube, the reaction mixture was then prepared (2 ml of the oil emulsion, 100 μl of the tested compound solution, and 200 μl of 10% $FeSO_4$ solution) and incubated under UV irradiation (h = 150–200 mm) during 150 min. The reference specimen was prepared similarly, using 100 μl of distilled water instead of the tested compound solution. After the incubation, 1 ml of 28% trichloroacetic acid solution was added, and the mixture was centrifuged during 10 min at 6000 rpm. 2 ml of supernatant was transferred into a large tube, 1 ml of 1% thiobarbituric acid solution was added, and the resulting sample was kept in a boiling water bath during 15 min. The solution absorbance was measured at 450 and 532 nm.

REFERENCES

1. Prosenko, A.E., Terakh, E.I., Pinko, P.I., Kandalintseva, N.V., Markov, A.F., Krysin, A.P., and Grigor'ev, I.A., *Nauka – Proizvod.*, 2004, vol. 73, no. 5, p. 18.
2. Arefrev, D.V., Belostotskaya, I.S., Volreva, V.B., Domnina, N.S., Komissarova N.L., Sergeeva, O.Yu., and Khrustaleva, R.S., *Russ. Chem. Bull.*, 2007, no. 4, p. 781.
3. Alekseeva, A.A., Smuseva. V.V., and Belenikina, S.A., Abstracts of Papers, *63th Open Science Conference of Students and Young Scientists of VolGMU,* Volgograd, 2005, p. 103.
4. Kosolapov, V.A., El'tsova, L.V., Tibir'kova, E.V., and Spasov, A.A., in *Sovremennaya innovatsionnaya meditsina – naseleniyu Volgogradskoi oblasti* (Contemporary Innovative Medicine to the Volgograd Region Inhabitants), Volgograd: VolGMU, 2008.
5. Kemeleva, E.A., Vasyunina, E.A., Sinitsinam O.I., Khomchenko, A.S., Gross, M.A., Kandalintseva, N.V., Prosenko, A.E., and Nevinskii, G.A., *Russ. J. Bioorg. Chem.*, 2008, vol. 34, no. 4, p. 499.
6. Khubaeva, T.O. and Zakaeva, R.Sh., *Russ. J. Gen. Chem.*, 2012, vol. 82, no. 3, pp. 446–454.
7. Pavlyuchenko, I.I., *Docroral (Med.) Dissertation*, Krasnodar, 2005.