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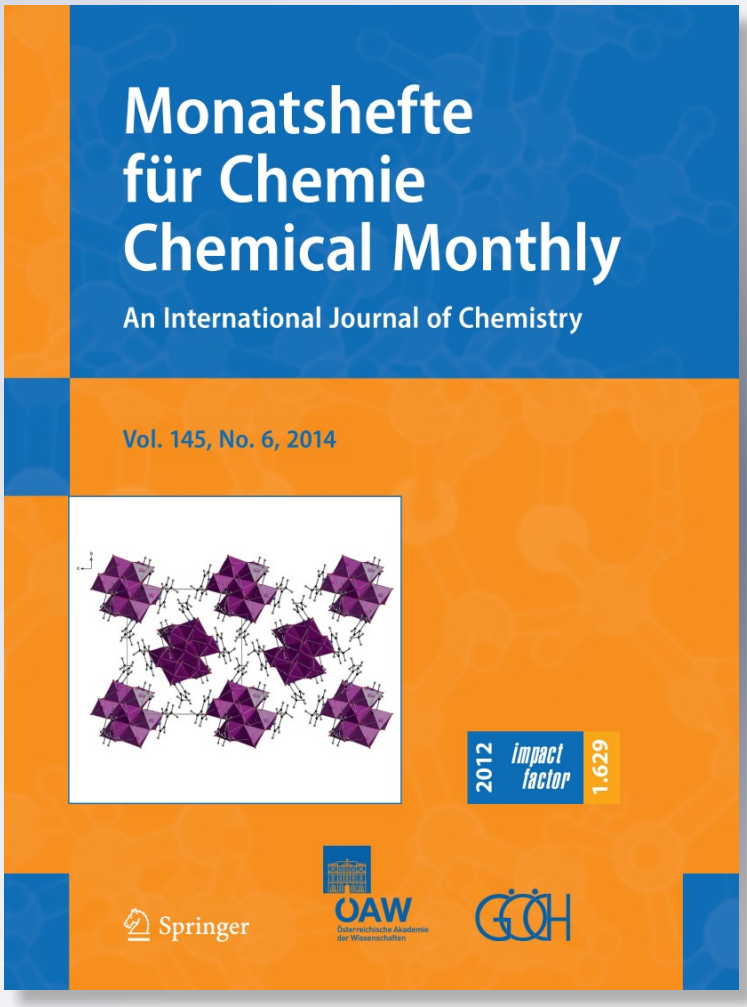

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ORIGINAL PAPER

2-Amino-5-alkylidenethiazol-4-ones as promising lipid peroxidation inhibitors

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Abstract 2-Amino-5-alkylidenethiazol-4-one represents a promising scaffold in medicinal chemistry and drug discovery. In the present study the antioxidant activity of 30 diverse 2-amino-5-alkylidenethiazol-4-ones was screened using a lipid peroxidation (LP) method. All compounds under study showed activity regardless of the substituent nature. However, several compounds exhibited a significant LP inhibition effect, which was in the range of that obtained with standard antioxidants. Compounds containing an (indol-3-yl)methylene group at position 5 of thiazol-4-one moiety and a six-membered ring as the 2-amino substituent showed inhibitory effects higher than 60 %. The most active compound, 5-benzylidene-2-morpholinothiazol-4(5*H*)-one, was investigated by means of ab initio calculations in order to clarify the most probable mechanism of antioxidant action. These calculations imply that electron transfer from the 2-amino-5-alkylidenethiazol-4-ones to the lipid alkoxyl, lipid peroxyl, or hydroxyl radicals could produce radical cations able to scavenge the lipid radicals and produce adducts, and ultimately terminate the reaction by proton transfer. Thus we propose the electron transfer (SET) mechanism as the most probable one that explains the observed inhibition of LP.

Keywords Ab initio calculations · Antioxidant activity · Heterocycles · Lipid peroxidation inhibitors · Redox reactions

J. Zvezdanovic · D. Cvetkovic · D. Markovic
Faculty of Technology, University of Niš, Bulevar oslobodjenja 124, 16000 Leskovac, Serbia

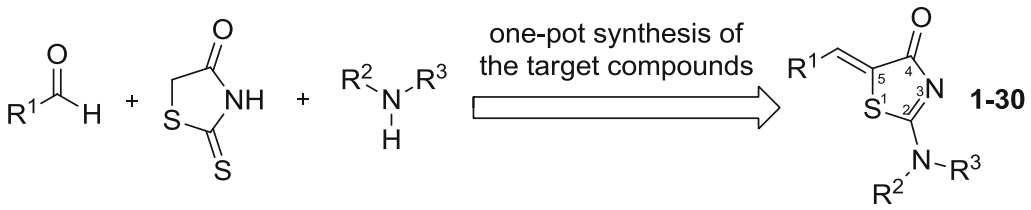
L. Daskalova · D. Yancheva
Institute of Organic Chemistry and Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Build. 9, 1113 Sofia, Bulgaria

M. Anderluh (✉)
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana, Slovenia
e-mail: marko.anderluh@ffa.uni-lj.si

A. Smelcerovic (✉)
Department of Chemistry, Faculty of Medicine, University of Niš, Bulevar Dr Zorana Djindjica 81, 18000 Niš, Serbia
e-mail: a.smelcerovic@yahoo.com

Introduction

Lipid peroxidation (LP) is mostly controlled by the action of antioxidants in vivo. Many biomolecules (and classes of biomolecules) serve as antioxidants, like enzymes, tocopherols (vitamin E), L-ascorbic acid (vitamin C), retinol (vitamin A), thiamin and riboflavin (vitamin B), flavonoids, etc. [1–3]. However it is worth noting that whatever method of initiation has been applied (i.e., independently of the operating external triggering mechanism; see the review by Girotti [4]) the chain reaction may be inhibited without involvement of any other scavenging species through recombination of the created lipid (L·) and lipid-peroxide radicals (LOO·). This makes up a self-inhibited LP, which constitutes a balance to the lipid auto-oxidation process. From the experiments performed in a "constrained environment" mimicking that of lipid membrane models—where mobility of the created radical species is highly limited by the cage effect, like in micelles [5–7] or in compressed lipid monolayers [8, 9]—it can be concluded that in (natural) membranes the high-density packed ordering of lipid molecules plays a crucial role in preventing LP chain propagation, not neglecting the role of other factors (antioxidants etc.).



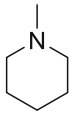
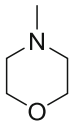
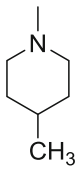
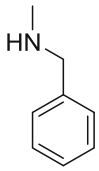
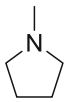
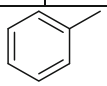
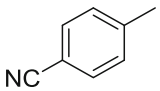
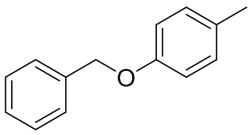
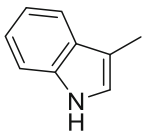
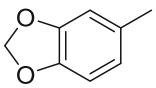
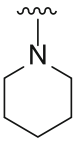
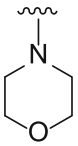
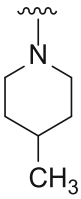
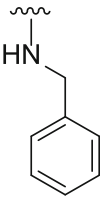
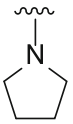
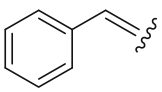
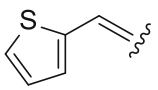
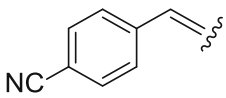
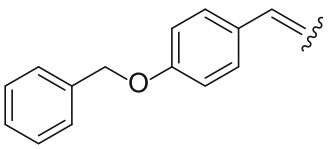
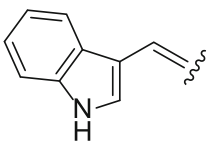
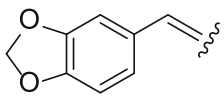
R^1 \ R^3R^2N					
	**1**	**2**	**3**	**4**	**5**
	**6**	**7**	**8**	**9**	**10**
	**11**	**12**	**13**	**14**	**15**
	**16**	**17**	**18**	**19**	**20**
	**21**	**22**	**23**	**24**	**25**
	**26**	**27**	**28**	**29**	**30**

Fig. 1 Synthesized 2-amino-5-alkylidene-thiazol-4-ones **1–30** [16]

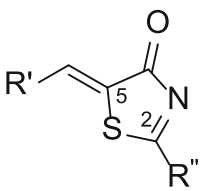
2-Amino-5-alkylidenethiazol-4-one is a privileged scaffold in drug discovery [10, 11] as its derivatives show a variety of biological activities, such as antimicrobial [12], antiviral [13], anti-inflammatory [14], and cardiotonic [15], to name a few. The attractiveness of this scaffold lies in the simplicity of its synthesis, as it can lead to variously trisubstituted derivatives and thus provide versatility for the development of potential drug candidates [11]. To simplify even further the availability of 2-amino-5-alkylidenethiazol-4-ones, we have developed a one-pot tandem reaction for their synthesis, and have synthesized a small focused library of the target compounds **1–30** (Fig. 1) [16].

Recently, we investigated the antimicrobial activity and cytotoxicity of compounds **1–30** [17]. Most of the compounds exhibited modest to significant antibacterial activity against *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus*, but not against *Salmonella typhimurium* and *Escherichia coli*. The important feature of the tested compounds is their low level of influence on cell viability, as tested by the HEK-293 metabolic activity

Table 1 Lipid peroxidation inhibition effect of studied 2-amino-5-alkylidenethiazol-4-ones (sample concentration 1 mg/cm^3) and selected antioxidants

Substituent at position 5	Substituent at position 2 Entry Lipid peroxidation inhibition effect/%					
		**1**	**2**	**3**	**4**	**5**
		22.95 ± 0.45	95.76 ± 0.97	31.73 ± 2.38	55.76 ± 1.01	31.03 ± 5.96
		**6**	**7**	**8**	**9**	**10**
		21.20 ± 1.06	63.06 ± 2.15	16.05 ± 0.01	38.11 ± 1.68	29.86 ± 2.35
		**11**	**12**	**13**	**14**	**15**
		36.35 ± 4.84	62.09 ± 1.83	52.70 ± 1.93	78.82 ± 1.13	33.75 ± 0.61
		**16**	**17**	**18**	**19**	**20**
		20.95 ± 2.34	30.64 ± 1.86	39.61 ± 3.46	94.13 ± 1.13	35.80 ± 0.50
		**21**	**22**	**23**	**24**	**25**
		83.26 ± 1.65	60.56 ± 1.37	89.36 ± 0.60	83.46 ± 2.04	27.23 ± 7.29
		**26**	**27**	**28**	**29**	**30**
		49.74 ± 8.48	10.77 ± 1.32	57.12 ± 1.06	37.00 ± 1.69	40.11 ± 9.65

Standard antioxidants	Sample concentration 0.01 mg/cm^3	Sample concentration 1 mg/cm^3
Trolox	38.53 ± 0.13	85.61 ± 2.05
Quercetin	43.68 ± 2.85	91.12 ± 0.36
Caffeic acid	69.09 ± 0.91	99.98 ± 2.52
L-Ascorbic acid	42.81 ± 0.40	99.90 ± 1.70



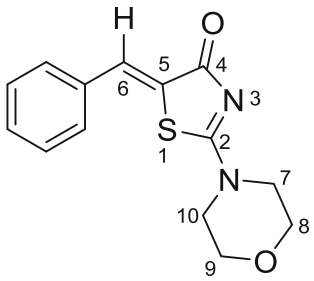


Fig. 2 Structure of compound **2**

assay. In the present study, the antioxidant activity of **1**–**30** was investigated using an LP method. An attempt to correlate the examined biological effects with the structure of the studied compounds was undertaken. We used the most active 2-amino-5-alkylidenethiazol-4-one derivative as a model compound to study the possible mechanisms of an antioxidant action.

Results and discussion

The LP inhibition effect of the studied 2-amino-5-alkylidenethiazol-4-ones **1**–**30** at a concentration of 1 mg/cm^3 ranged between 11 and 96 % (Table 1). 5-Benzylidene-2-morpholinothiazol-4(5*H*)-one (compound **2**; Fig. 2) showed the most potent inhibitory effect. All compounds containing a (indol-3-yl)methylene group at position 5 of the thiazol-4-one moiety and a six-membered ring as the 2-amino substituent showed inhibitory effects higher than 60 %. The studied compounds containing pyrrolidin-1-yl as the 2-amino substituent showed inhibitory effects under 41 %. At the studied concentration of 1 mg/cm^3, compounds **2**, **14**, **19**, **21**, **23**, and **24** showed stronger inhibitory effects than conventionally used standard compounds (trolox, quercetin, caffeic acid, and L-ascorbic acid) at concentrations of 0.01 mg/cm^3, and comparable effects to standard antioxidants at a concentration of 1 mg/cm^3 (see Table 1).

One possible mechanism by which the antioxidants can deactivate a free radical is H atom abstraction (HAT mechanism) [18–21]:

$$\text{Antiox-}H \rightarrow \text{Antiox}^{\cdot} + \text{H}^{\cdot}\,(\text{HAT})$$

The efficacy of the antioxidant to react via HAT is characterized by the bond dissociation enthalpy (BDE). Higher stability of Antiox-H, i.e., lower BDE values, corresponds to a good antioxidant capacity of Antiox-H.

Another possible mechanism is electron transfer (SET mechanism), in which a radical cation is first formed followed by deprotonation [18, 21–24]:

$$\text{Antiox-}H \rightarrow \text{Antiox}{-}H^{+\cdot} + e^{-}\,(\text{SET})$$
$$\text{Antiox}{-}H^{+\cdot} \rightarrow \text{Antiox}^{\cdot} + \text{H}^{+}$$

For evaluation of the reactivity via SET, the ionization potential (IP) is used. A lower IP implies an easier extraction of the electron. As all compounds in the studied series of 2-amino-5-alkylidenethiazol-4-ones show activity regardless of the substituent nature, the 5-alkylidenethiazol-4-one moiety could be recognized as the active center. The only possible site for H abstraction in the 5-alkylidenethiazol-4-one moiety is C6 (Fig. 2).

The feasibility of the HAT mechanism was estimated by calculating the reaction enthalpy of radical formation by abstraction of a hydrogen atom from this position. We used the most active 2-amino-5-alkylidenethiazol-4-one derivative **2** as a model compound, which showed no cytotoxic effects on the HEK-293 cell line at a concentration of 25 μg/cm^3 [17]. According to the B3LYP/6-311++G** calculations, the corresponding BDE value is 416 kJ/mol. For comparison, free radicals of the type LOO· typically display a BDE of about 367 kJ/mol [25]. Thus, an effective chain-breaking antioxidant that could prevent LP should have a lower BDE value. Well-known antioxidants that react via HAT are α-tocopherol, curcumin, epigallocatechin gallate, and caffeic acid with BDE values of 327 [18], 357 [26], 297 [21], and 321 kJ/mol [27]. In the present case, the calculated BDE value is substantially higher and excludes HAT as a probable mechanism of action.

On the other hand, the 2-amino-5-alkylidenethiazol-4-ones may be envisaged as electron-donating compounds because they might undergo oxidation via a stepwise mechanism; an initial electron transfer (SET mechanism) could produce a radical cation able to scavenge the lipid alkoxyl (LO·), lipid peroxyl (LOO·), or hydroxyl (·OH) radicals, followed by formation of an intermediate adduct and termination by a proton transfer (Fig. 3).

An investigation of the structure of the radical cations and adducts of **2** formed in the course of the antioxidant action according to Fig. 3 was undertaken by including a methoxy radical to represent the lipid. The first step in the reaction is formation of the actual radical scavengers—the radical cations of the studied compounds. The optimized geometry of the radical cations shows substantial shortening of the S1–C5 bond and lengthening of C5–C6. The analysis of the spin densities over the atoms indicates that half of the unpaired electron is localized in the fragment S1–C5–C6 and could be represented by the resonance structures shown in Fig. 4.

Thus, the most reactive sites in the attack of the lipid radicals would be S1, C5, and C6. The possibility to form adducts by addition of a methoxy radical at these sites, as well as at C2, was studied by optimizing the geometry of

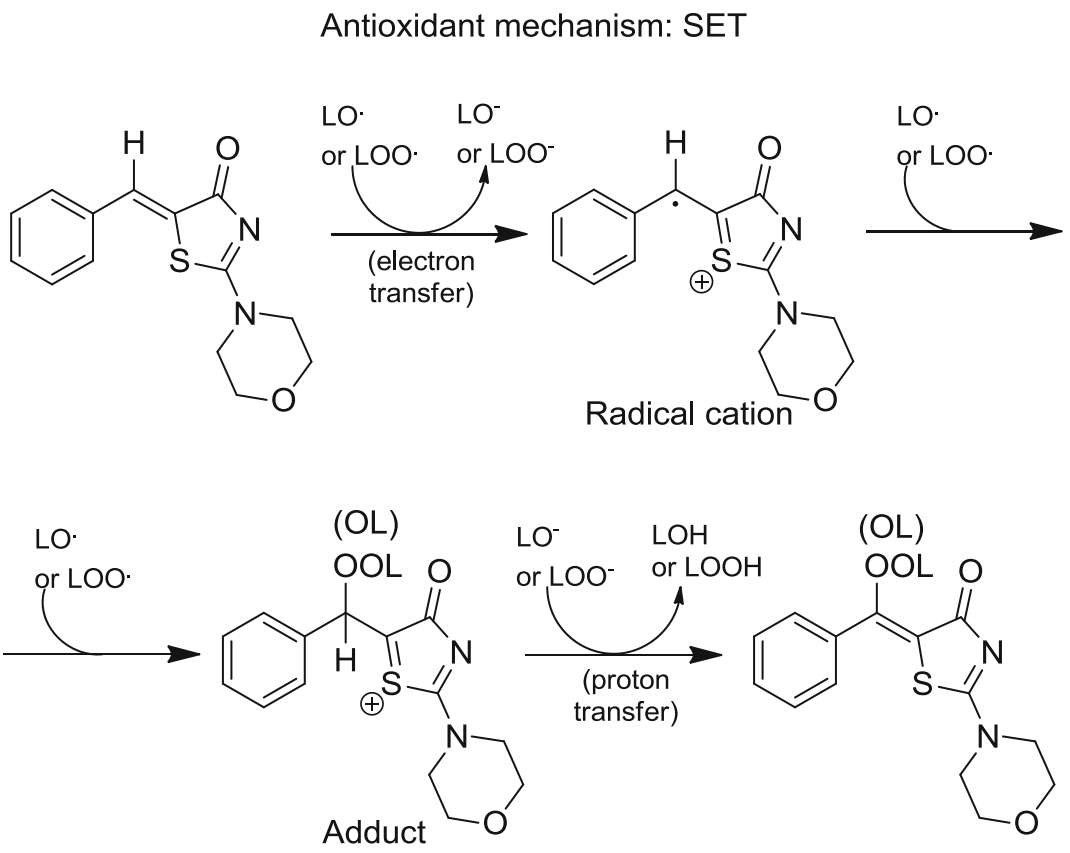


Fig. 3 Hypothetical mechanism of antioxidant action of 2-amino-5-alkylidenethiazol-4-ones

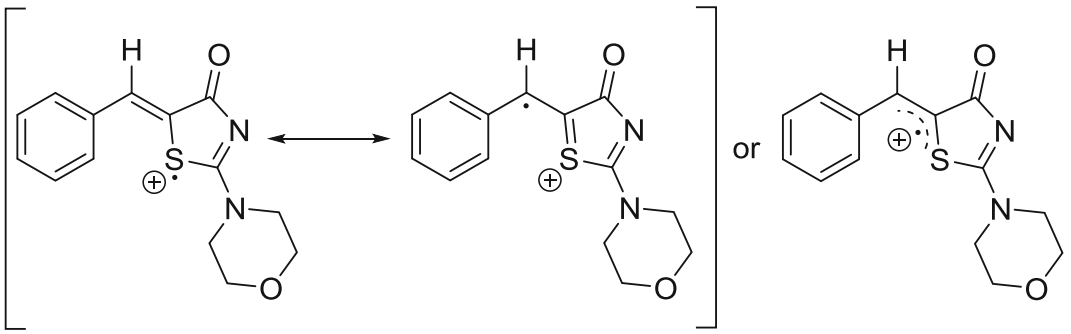


Fig. 4 Resonance structures of the peroxyl scavenger (radical cation)

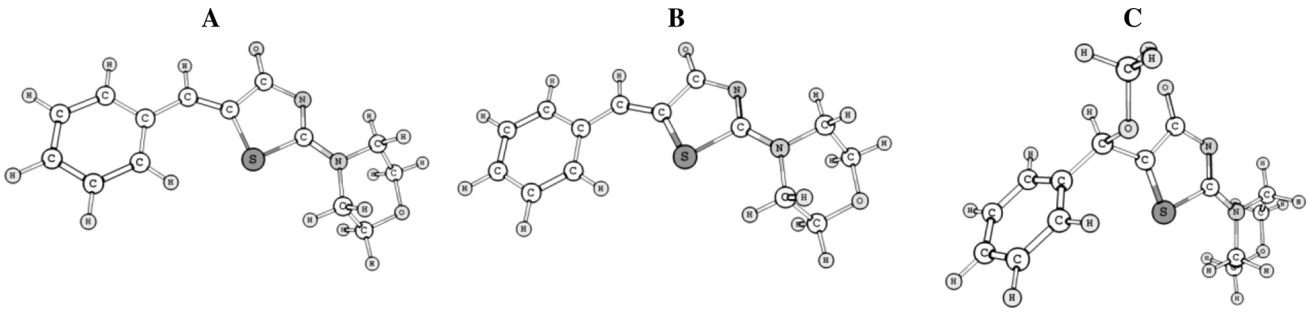


Fig. 5 Structures of neutral molecule (**a**), radical cation (**b**), and the proposed adduct at C6 (**c**) of **2**. The structures were generated with Chemcraft software [35]

the relevant cation products at the B3LYP/6-311++G** level. According to the calculated zero-point vibrational energy (ZPVE)-corrected total energies, addition at C6 seems the most favorable. Unlike the radical cation, which retains the coplanar orientation of the phenyl ring and 5-alkylidenethiazol-4-one moiety, the proposed adduct has tetrahedral structure due to the sp^3 hybridization of C6 (Fig. 5). In the final step of the proposed antioxidant reaction, after transferring a proton to the lipid anion, the molecules would restore their planarity.

A similar mechanism of antioxidant activity has been proposed for pyrrolopyrimidines, which were described as effective in vitro and in vivo antioxidants possessing neuroprotective activity in brain injury and ischemia models [28]. In the latter case, the authors found evidence for the validity of the suggested scheme by electrochemical oxidation under very mild conditions, in methanol or acetic acid, affording the expected methoxy and acetoxy adducts. Another antioxidant whose reactions with oxygenated free radicals are believed to involve adducts formation is caffeine [29, 30].

Finally, it is worth noting that this study does not take into account the influence that the structure of the lipids might have toward the antioxidant ability of the used compounds. The fatty acid branches of the lipids with their "anticonjugated structure" may play a crucial role in facilitating the HAT mechanism—through an easier abstraction of their allylic and doubly-allylic H atoms [5, 7, 31]—by lowering the related BDE values, which would permit more efficient triggering of the LP chain mechanism. However, because the lipid structural effects are not dominant in solution—compared to the organized models and natural membranes—they were not particularly considered in this study.

Conclusions

2-Amino-5-alkylidenethiazol-4-ones were documented to be promising LP inhibitors. The antioxidant activity of some of the studied compounds was comparable to that of standard antioxidants (see results for **2**, **19**, trolox, quercetin, caffeic acid, and L-ascorbic acid at a concentration of 1 mg/cm^3).

All the compounds in the studied series showed antioxidant activity regardless of the substituent nature, which implies that the 2-amino-5-alkylidenethiazol-4-one moiety is the active center of the antioxidant action. However, the mechanism relying on the direct H abstraction (HAT) is anticipated to be energetically unfavored because of the lack of functional groups, which would readily scavenge lipid peroxyl radicals. This is supported by the high BDE value (416 kJ/mol) calculated for **2**, the most active compound in the studied series, which is far from the typical range of 295–360 kJ/mol exhibited by well-known antioxidants reacting via HAT.

Electron transfer from the 2-amino-5-alkylidenethiazol-4-ones to the lipid alkoxyl, lipid peroxyl, or hydroxyl radicals (SET mechanism) would produce radical cations able to scavenge the lipid radicals, to form adducts, and ultimately terminate the reaction by proton transfer. The structure and spin density population analysis, performed at the B3LYP/6-311++G** level for the cation of **2**, showed that the most reactive sites in the attack of the lipid radicals would be atoms S1, C5, and C6. On the basis of the relative stability of the corresponding methoxy adducts, addition at C6 is the most favorable.

Taking into account the electron-donating properties of the 2-amino-5-alkylidenethiazol-4-ones and the high calculated BDE value of **2**, SET according to the suggested scheme is the most probable mechanism of antioxidant action of these compounds.

Experimental

Phospholipids (Phospholipon® 90; PL90) were gifted by Phospholipid GMBH, Cologne, Germany. According to the manufacturer the mixture content of PL90 is phosphatidylcholine 98 %, lyso-phosphatidylcholine 2.1 %; the fatty acid composition is palmitic acid 12 ± 2 %, stearic acid 3 ± 1 %, oleic acid 10 ± 3 %, linoleic acid 66 ± 5 %, linolenic acid 5 ± 2 %; peroxide value maximum 1.3. PL90 was kept in the dark to prevent the photooxidation process. Thiobarbituric acid (TBA), 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), and standards of trolox, quercetin, caffeic acid, and L-ascorbic acid were obtained from Sigma Aldrich. Compounds **1–30** were prepared and characterized according to Ref. [16].

Thiobarbituric acid–malondialdehyde test

Lipid peroxidation, as well as its inhibition in the presence of the studied 2-amino-5-alkylidenethiazol-4-ones, was measured by using the thiobarbituric acid–malondialdehyde (TBA–MDA) test [2, 32]. This method is based on the MDA (secondary product of LP) reaction with TBA to obtain a red colored complex with maximum absorption at 530 nm. The reaction mixture of 0.3 cm^3 contained a methanol solution of PL90 (1×10^{-2} mol/dm^3) and methanol solutions of selected synthesized compounds (1 g/dm^3) in 2:1 (v/v) ratio. Lipid peroxidation was initiated by using 0.2 cm^3 (2.2×10^{-2} mol/dm^3) aqueous solution of hydrophilic thermal initiator of LP [2,2′-azobis(2-methylpropionamidine) dihydrochloride, AAPH] during a time period of 3 h at 40 °C. Immediately after this

period 1 cm^3 of aqueous trichloroacetic acid (5.5 %), followed by 0.5 cm^3 of TBA (4.2×10^{-2} mol/dm^3 in 5×10^{-2} mol/dm^3 NaOH) and BHT (1×10^{-3} mol/dm^3) were added to the reaction mixture. The mixture was incubated for 10 min at 65 °C in the dark, and centrifuged for 5 min at 13,800 rpm. The TBA–MDA complex absorbances in the supernatant read at 530 nm were used to calculate the inhibition percentage of LP by using the following equation:

$$\text{Inhibition of lipid peroxidation } (\%) = 100 \times (A_c - A_s)/(A_c - A_b)$$

where A_c is the absorbance of control (solution of pure PL90) which is treated with the AAPH solution, as well as the TBA solution, A_s the absorbance of sample (PL90/**1**–**30**, sample mixture) which is treated with the AAPH and TBA solution, and A_b is the absorbance of blank [solution of pure PL90 which is not treated with AAPH, but treated with TBA solution (monitoring MDA level in the lipid before LP initiation by AAPH)]. The same experiments were done by using standard compounds (at a concentration of 0.01 mg/cm^3) such as trolox, a synthetic antioxidant, and quercetin, caffeic acid, and L-ascorbic acid as natural antioxidants.

Computational details

All theoretical calculations were performed using the Gaussian 09 package [33] of programs. Geometry and vibrational frequencies of the species studied were performed by analytical-based gradient technique without any symmetry constraint. The geometries of *Z*- and *E*-isomers of the studied compound were fully optimized using density functional theory (DFT), employing the B3LYP (Becke's three-parameter non-local exchange) [34] and Lee et al. correlation [33] potentials and the 6-311++G** basis set. The *Z*-isomers were found to be more stable which is in accordance with the stereochemistry established by NMR data [16]. The optimized structures were further characterized by analytic computations of harmonic vibrational frequencies at the same level. For optimization of the geometry of the radical at the UB3LYP/6-311++G** level, only the *Z*-form was considered. BDEs were calculated according the equations given by Klein et al. [18]:

$$\text{BDE} = H(\text{Antiox}^{\cdot}) + H(\text{H}^{\cdot}) - H(\text{Antiox-}H)$$

The total energy of the hydrogen atom, used in the BDE calculations is −0.502257 hartree. The calculated enthalpy of the proton, $H(H^+)$, is 6.197 kJ/mol; the enthalpy of the electron, $H(e^-)$, is 3.145 kJ/mol. All reaction enthalpies were calculated for 298 K.

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References

1. Saija A, Scalese M, Lanza M, Marzzullo D, Bonina F, Castelli F (1995) Free Radic Biol Med 19:481
2. Choi CW, Kim SC, Hwang SS, Choi BK, Ahn HJ, Lee MY, Park SH, Kim SK (2002) Plant Sci 163:1161
3. Heim KE, Tagliaferro AR, Bobilya DJ (2002) J Nutr Biochem 13:572
4. Girotti AW (2001) J Photochem Photobiol B 6:103
5. Marković DZ, Durand T, Patterson LK (1990) Photochem Photobiol 51:389
6. Markovic DZ, Patterson LK (1993) Photochem Photobiol 58:329
7. Markovic DZ (2004) J Serb Chem Soc 69:107
8. Markovic DZ (2001) Collect Czech Chem Commun 66:1603
9. Markovic D (2006) J Serb Chem Soc 71:349
10. Mukhopadhyay C, Ray S (2011) Tetrahedron 67:7936
11. Pulici M, Quartieri F (2005) Tetrahedron Lett 46:2387
12. Hu Y, Helm JS, Chen L, Ginsberg C, Gross B, Kraybill B, Tiyanont K, Fang X, Wu T, Walker S (2004) Chem Biol 11:703
13. Abdel-Ghani E (1999) J Chem Res (S) 174
14. Song Y, Connor DT, Doubleday R, Sorenson RJ, Sercel AD, Unangst PC, Roth BD, Gilbertsen RB, Chan K, Schrier DJ, Guglietta A, Bornemeier DA, Dyer RD (1999) J Med Chem 42:1151
15. Andreani A, Rambaldi M, Leoni A, Locatelli A, Bossa R, Chiericozzi M, Galatulas I, Salvatore G (1996) Eur J Med Chem 31:383
16. Anderluh M, Jukic M, Petric R (2009) Tetrahedron 65:344
17. Jukič M, Đorđević A, Lazarević J, Gobec M, Šmelcerović A, Anderluh M (2013) Mol Divers 17:773
18. Klein E, Lukes V, Ilcin M (2007) Chem Phys 336:51
19. Burton GW, Doba T, Gabe EJ, Hughes L, Lee FL, Prasad L, Ingold KU (1985) J Am Chem Soc 107:7053
20. de Heer MI, Mulder P, Korth H-G, Ingold KU, Lusztyk J (2000) J Am Chem Soc 122:2355
21. Wright JS, Johnson ER, DiLabio GA (2001) J Am Chem Soc 123:1173
22. Musialik M, Litwinienko G (2005) Org Lett 7:4951
23. Vafiadis AP, Bakalbassis EG (2005) Chem Phys 316:195
24. Rimarčík J, Lukeš V, Klein E, Ilčin M (2010) J Mol Struct (Theochem) 952:25
25. Berkowitz J, Ellison GB, Gutman D (1994) J Phys Chem 98:2744
26. Wright JS (2002) J Mol Struct (Theochem) 591:207
27. Chen W, Guo P, Song J, Cao W, Bian J (2006) Bioorg Med Chem Lett 16:3582
28. Hall ED, Andrus PK, Smith SL, Fleck TJ, Scherch HM, Lutzke BS, Sawada GA, Althaus JS, Vonvoigtlander PF, Padbury GE, Larson PG, Palmer JR, Bundy GL (1997) J Pharmacol Exp Ther 281:895
29. Leon-Carmona JR, Galano A (2011) J Phys Chem B 115:4538
30. Dalmazio I, Santos LS, Lopes RP, Eberlin MN, Augusti R (2005) Environ Sci Technol 39:5982
31. Markovic DZ, Patterson LK (1989) Photochem Photobiol 49:531
32. Fernandez J, Perez-Alvarez JA, Fernindez-Lopez JA (1997) Food Chem 59:345
33. Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Scalmani G, Barone V, Mennucci B, Petersson GA, Nakatsuji H, Caricato M, Li X, Hratchian HP, Izmaylov AF,

Bloino J, Zheng G, Sonnenberg JL, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Vreven T, Montgomery JA, Peralta JE, Ogliaro F, Bearpark M, Heyd JJ, Brothers E, Kudin KN, Staroverov VN, Kobayashi R, Normand J, Raghavachari K, Rendell A, Burant JC, Iyengar SS, Tomasi J, Cossi M, Rega N, Millam JM, Klene M, Knox JE, Cross JB, Bakken V, Adamo C, Jaramillo J, Gomperts R, Stratmann RE, Yazyev O, Austin AJ, Cammi R, Pomelli C, Ochterski JW, Martin RL, Morokuma K, Zakrzewski VG, Voth GA, Salvador P, Dannenberg JJ, Dapprich S, Daniels AD, Farkas O, Foresman JB, Ortiz JV, Cioslowski J, Fox DJ (2009) Gaussian 09, revision A1. Gaussian Inc, Wallingford

34. Stephens PJ, Devlin FJ, Chabalowski CF, Frisch MJ (1994) J Phys Chem 98:11623
35. ChemCraft version 1.7 (2012) http://www.chemcraftprog.com. Accessed 2 Feb 2014