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Novel (3,5-di-*tert*-butyl-2-hydroxy-phenylcarbamoyl)-alkanoic acids as potent antioxidants

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Abstract—A series of novel phenolic antioxidants of amphiphilic structure has been synthesized. Investigations into the influence of aliphatic spacer length and nature of a hydrophilic anchor on the antioxidant activity allowed elucidating certain structure requirements for the membrane-addressed antioxidant designing.

Uncontrolled oxidative damage of biomolecules is one of the important factors causing occurrence and development of numerous degenerative diseases (rheumatoid arthritis, atherosclerosis, cardiac infarction, stroke, Alzheimer's and Parkinson's diseases, etc.).[1] Therefore, mitigation and removal of the oxidative stress by means of using natural[2] and synthetic antioxidants[3] is regarded as a practicable strategy in prevention and treatment of the named diseases.[4] Thus, there is evidence of a protective effect of α-tocopherol in cases of reperfusional syndrome[5a] and atherosclerosis.[5b] A synthetic antioxidant of phenolic nature, BO-653 (**1**) (Fig. 1), which has been developed by means of a rational designing strategy,[6a] manifests a marked anti-atherogenic effect in vivo[6b] and is proposed as an agent for prevention of atherosclerosis.[6c]

A very promising approach is also combining antioxidant properties with other types of biological activity within a single chemical structure. For example, piperazine, AM-36 (**2**) (Fig. 1), being simultaneously a sodium channel blocker and an antioxidant, diminishes the extent of cardiac infarction and decreases the level of reactive oxygen species within the ischemic tissue.[7a] As shown by the studies performed in animal models, a neuronal synthase inhibitor, BN 80933 (**3**) (Fig. 1), containing an α-tocopherol residue in its molecular structure, decreases brain damage in cases of cranial injuries and ischemic conditions.[7b] In that way, antioxidants are promising therapeutic agents, and elucidation of factors that optimize their anti-radical activity appears to be an important target for investigation.

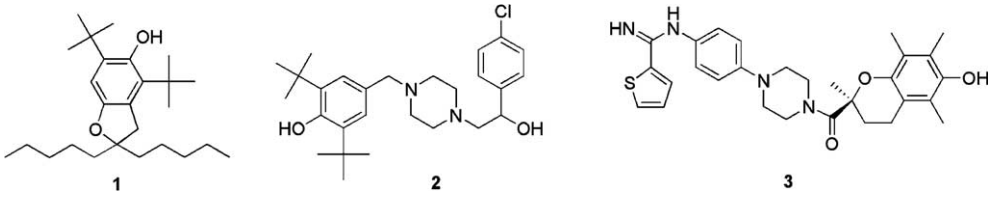

Figure 1. Drug candidates bearing antioxidant properties.

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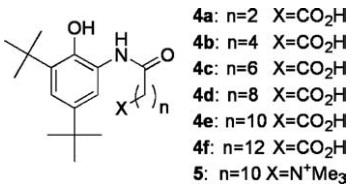


Figure 2. Chemical structures of new membrane-addressed antioxidants.

In our previous paper,[8] we have reported about synthesizing a new amphiphilic antioxidant, 11-(3,5-di-*tert*-butyl-2-hydroxyphenylcarbamoyl)undecanoic acid (**4e**) (Fig. 2). Designing of this lipid peroxidation (LPO) inhibitor has been performed on the basis of the membrane-addressing concept. The essence of this concept is introduction of a hydrophilic group into the terminal part of molecular side chain of an antioxidant. The hydrophilic group plays the role of an 'anchor' on the lipid membrane surface, while the phenolic moiety penetrates deeply into the lipid bilayer, where just the LPO chain reaction takes place. Owing to such an 'addressed' delivery of the phenolic pharmacophore to a definite area of the membrane, a significant increase of inhibitory efficacy of membrane-addressed antioxidants with respect to LPO is observed, as compared to their nonamphiphilic analogues.[8]

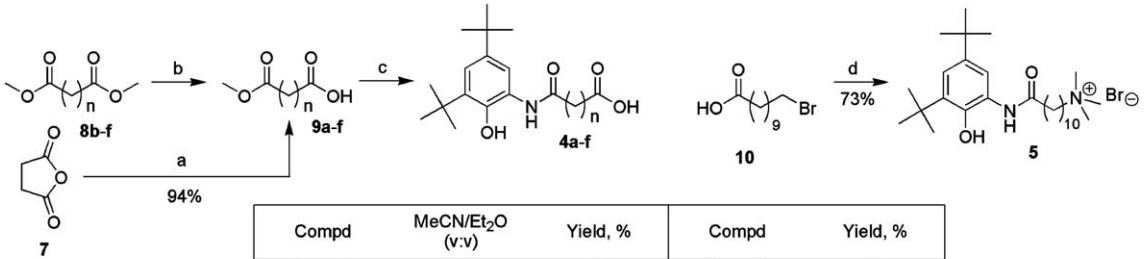
It should be expected that the nature of terminal substituent and length of the aliphatic spacer between the hydrophilic anchor and the phenolic pharmacophore would exert a substantial influence on activity of membrane-addressed antioxidants. Besides, there are negatively charged phosphate groups on the surface of a membrane, therefore, it should be of interest to follow changes in antioxidant activity on changing from an anionic anchor to a cationic one. In this paper, we describe syntheses of homologues **4a**–**f** and an analogue **5** (Fig. 2) with a cationic hydrophilic group, and discuss the structure–antioxidant activity relationship in the series of compounds prepared.

Syntheses of homologous acids **4a**–**f** have been performed according to an approach that we have described earlier[8] (Scheme 1). 2-Amino-4,6-di-*tert*-butylphenol (**6**), readily obtained from catechol, was regioselectively acylated with acyl chlorides of the respective dicarboxylic acid monomethyl esters **9a**–**f**. Treatment of the obtained amides with LiOH in aqueous methanol, followed by acidification of the reaction mixture with 2 N HCl, led to the target acids **4a**–**f**[9] in high yields. Monomethylsuccinate (**9a**) was synthesized in high yields by treating succinic anhydride with methanol at room temperature. Monomethyl esters **9b**–**f** were prepared by monohydrolysis of readily available dimethyl esters **8b**–**f** in the system KOH–MeOH–MeCN–Et_2O (Scheme 1). The modified procedure that we have developed, which consisted in increasing v/v percentage of a more polar solvent (MeCN) in cases of more lipophilic diesters, **9e**–**f**, or increasing that of less polar solvent (Et_2O) in cases of less lipophilic diesters, **9b**,**c**, resulted in higher yields of products **9b**–**f**, as compared to the original procedure.[10]

Compound **5**[9] was prepared by acylation of aminophenol **6** with 11-bromoundecanoic acid acyl chloride followed by quaternization with trimethylamine in the system DMFA–$CHCl_3$–EtOH for 5 days (Scheme 1).

Antioxidant activity of compounds **4a**–**f** and **5** was evaluated by their influence on the radiation-induced peroxidation of phosphatidyl choline in multilamellar liposomes.[11] The extent of LPO was determined by the accumulation of conjugated diene products (CDP)[12] and 2-thiobarbituric acid reactive substances (TBARS)[13] (Table 1).

Lipophilicity (log *P*) is also an important characteristic of membrane-addressed antioxidants, because log *P* characterizes affinity of a compound toward the lipid phase. The log *P* values for compounds **4a**–**f** and **5** were calculated by means of CLOGP[14] (Table 1). The log *P* values for the acids, **4a**–**f**, correspond to anionic forms of the compounds, because just anionic forms will prevail at physiologic pH values.



Compd	$MeCN/Et_2O$ (v:v)	Yield, %	Compd	Yield, %
9a, n=2	—	94	**4a**, n=2	82
9b, n=4	1:1.5	71	**4b**, n=4	85
9c, n=6	1:1.2	75	**4c**, n=6	80
9d, n=8	1:1	85	**4d**, n=8	88
9e, n=10	1.2:1	80	**4e**, n=10	85
9f, n=12	1.5:1	79	**4f**, n=12	88

Scheme 1. Reagents and conditions: (a) MeOH (50 equiv), rt, 12 h. (b) KOH (1.15 equiv), MeOH–MeCN–Et_2O, 0 °C, 54 h. (c) (i) $(COCl)_2$ (1.1 equiv), toluene, rt, 4 h; (ii) **6** (1 equiv), Et_3N (1.1 equiv), THF, 0 °C → rt, 30 min; (iii) LiOH (5 equiv), MeOH–H_2O, 5 °C, 20 h; (iv) HCl 2 N. (d) (i) $(COCl)_2$ (1.1 equiv), toluene, rt, 4 h; (ii) **6** (1 equiv), Et_3N (1.1 equiv), THF, 0 °C → rt, 30 min; (iii) Me_3N (5 equiv, 20 wt% in EtOH), DMFA–$CHCl_3$–EtOH, rt, 120 h.

Table 1. Antioxidant activity and log *P* of compounds **4a–f** and **5**

Compd[a]	Inhibition of CDP formation[b] (%)		Inhibition of TBARS formation[b] (%)		Calcd log *P*[c]
	1.4 kGy	2.4 kGy	1.4 kGy	2.4 kGy	
4a, $n = 2$	50.2 ± 2.2	44.6 ± 2.3	51.8 ± 2.2	34.5 ± 3.3	1.10
4b, $n = 4$	56.9 ± 3.0	48.0 ± 2.8	59.0 ± 2.8	42.2 ± 2.4	0.47
4c, $n = 6$	62.1 ± 1.5	55.2 ± 4.1	63.4 ± 3.0	45.0 ± 2.0	1.50
4d, $n = 8$	75.7 ± 2.4	72.7 ± 3.6	79.7 ± 3.3	66.9 ± 3.6	2.56
4e, $n = 10$	53.1 ± 3.1	47.5 ± 3.4	67.5 ± 3.7	57.3 ± 2.1	3.62
4f, $n = 12$	43.5 ± 2.0	43.0 ± 2.0	43.4 ± 3.5	53.7 ± 1.9	4.67
5, $n = 10$	38.2 ± 2.8	35.5 ± 1.7	38.1 ± 2.4	36.7 ± 3.1	2.56
α-T	20.6 ± 3.0	21.8 ± 3.1	24.6 ± 3.4	20.4 ± 2.7	12.0

[a] The investigated compounds and α-tocopherol (a reference compound) were taken in the 0.1 mM concentration; the phosphatidyl choline concentration was 20 mM.

[b] Percent inhibition with respect to a blank experiment, without antioxidant. The confidence interval was calculated for $n = 3$, $p = 0.95$ using Student's *t*-distribution.

[c] The log *P* values of compounds **4a–f** were calculated for anionic forms.

Experimental results obtained in the liposomal model of LPO (Table 1) show that antioxidant activity of the compounds prepared depends to a significant extent on the aliphatic spacer length and nature of the membrane anchor. On examining changes in antioxidant properties of homologues **4a–f**, one can mark out a range of increased activity (compounds **4a–d**), a range of decreased activity (compounds **4d–f**), and a peak of activity corresponding to compound **4d**.

Such character of the structure–activity relationship can be explained from the standpoint of the membrane-addressing concept. In cases of short-chain homologues, the immersion depth of phenolic moiety into the membrane is small, and the antioxidant pharmacophore is situated above the membrane area where LPO takes place (methylene groups of the 1,4-pentadiene system belonging to polyunsaturated acid residues). Therefore, in the case of a short spacer, protective role of membrane-addressed antioxidants consists mainly in neutralization of radical species that penetrate into the membrane from the aqueous phase and initiate LPO; hence, antioxidant activity in this case is not particularly high. When the side chain length is gradually built up, the radical-scavenging part moves closer to the region where free-radical LPO processes take place, and antioxidant activity increases. With a spacer length of eight methylene units (compound **4d**), maximum activity is observed. To all appearances, such a spacer provides localization of the phenolic pharmacophore just within the area of development of free-radical LPO processes causing damage to the lipid membrane (C_7–C_{11} atoms of polyunsaturated fatty acid residues). In this case, along with neutralization of radicals initiating the LPO process, compound **4d** breaks up the chain of its development most efficiently, and this produces a high antiperoxidant effect. Further increase of the spacer length leads to a gradual decrease in activity, because the radical-scavenging region falls through into the depth of the lipid bilayer. Antioxidants now practically do not protect the membrane any longer from radical species penetrating from the aqueous phase. However, since the aliphatic spacer is a flexible structure, and the phenolic hydroxyl group can reside for sometime within the area of LPO occurrence, compounds **4e–f** still retain a sufficient inhibitory activity.

An illustrative example is comparison of activities of compounds **4a** and **4b** (Table 1). The anion of succinic acid derivative **4a** is more lipophilic than the anion of compound **4b** owing to the possibility of intramolecular hydrogen bond formation. At the same time, amide **4a** is inferior to compound **4b** as to antioxidant activity. This indicates that the aliphatic spacer length is a more important parameter determining inhibitory properties of an antioxidant than lipophilicity.

Quaternary ammonium salt **5**, with its side chain length close to an optimum ($n = 10$) and having a considerable lipophilicity, is, nevertheless, the least active of the group of antioxidants studied. This may be explained by the circumstance that compound **5** causes disruption of the lipid bilayer structure, leading to intensification of LPO.

In conclusion, synthesis of new membrane-addressed antioxidants **4a–f** and **5** has been described in this paper, and structure–antioxidant activity relationship in the series of compounds prepared has been discussed. The side chain consisting of eight methylene groups has been shown to provide maximum antioxidant activity. It has also been demonstrated that replacement of a carboxyl group in an amphiphilic antioxidant structure by a quaternary nitrogen leads to a considerable deterioration of inhibitory properties. As a result of this work, an effective antioxidant ($I_{\mathbf{4d}}/I_{\mathbf{\alpha\text{-}T}} = 3.5$) has been found that can be regarded as a primary structure in designing drug products for prevention and treatment of diseases caused by the oxidative stress.

Acknowledgements

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References and notes

1. (a) Cockrane, C. G. *Am. J. Med.* **1991**, *91*(Suppl. 3C), 23S; (b) Halliwell, B. *Drugs* **1991**, *42*, 569; (c) Rice-Evans, C. A.; Diplock, A. T. *Free Radical Biol. Med.* **1993**, *15*, 77; (d) *Free Radicals in Biology and Medicine*, 3rd ed.; Halliwell,

B., Gutteridge, J. M. C., Eds.; Oxford University Press: Oxford, 1999.
2. Bowry, V. R.; Ingold, K. U. *Acc. Chem. Res.* **1999**, *32*, 27.
3. (a) Hussain, H. H.; Babic, G.; Durst, T.; Wright, J. S.; Flueraru, M.; Chichirau, A.; Chepelev, L. L. *J. Org. Chem.* **2003**, *68*, 7023; (b) Wijtmans, M.; Pratt, D. A.; Valgimigli, L.; DiLabio, G. A.; Pedulli, G. F.; Porter, N. D. *Angew. Chem.* **2003**, *115*, 4506.
4. (a) Saunders, R. D.; Dugan, L. L.; Demediuk, P.; Means, E. D.; Horrocks, L. A.; Anderson, D. K. *J. Neurochem.* **1987**, *49*, 24; (b) Marcinik, G.; Petty, M. A. *Drugs Future* **1996**, *21*, 1037.
5. (a) Venditti, P.; Masullo, P.; Agnisola, C.; Di Meo, S. *Life Sci.* **2000**, *66*, 697; (b) Stephens, N. G.; Parsons, A.; Schofield, P. M.; Kelly, F.; Cheeseman, K.; Mitchinson, M. J.; Brown, M. J. *Lancet* **1996**, *347*, 781.
6. (a) Noguchi, N.; Iwaki, Y.; Takahashi, M.; Komuro, E.; Kato, Y.; Tamura, K.; Cynshi, O.; Kodama, T.; Niki, E. *Arch. Biochem. Biophys.* **1997**, *342*, 236; (b) Cynshi, O.; Kawabe, Y.; Suzuki, T.; Takashima, Y.; Kaise, H.; Nakamura, M.; Ohba, Y.; Kato, Y.; Tamura, K.; Hayasaka, A.; Higashida, A.; Sakaguchi, H.; Takeya, M.; Takanishi, K.; Inoue, K.; Noguchi, N.; Niki, E.; Kodama, T. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 10123; (c) Tamura, K.; Kato, Y.; Ishikawa, A.; Kato, Y.; Himori, M.; Yoshida, M.; Takashima, Y.; Suzuki, T.; Kawabe, Y.; Cynshi, O.; Kodama, T.; Niki, E.; Shimizu, M. *J. Med. Chem.* **2003**, *46*, 3083.
7. (a) Callaway, J. K.; Knight, M. J.; Watkins, D. J.; Beart, P. M.; Jarrott, B. *Stroke* **1999**, *30*, 2704; (b) Chabrier, P.-E.; Auguet, M.; Spinnewyn, B.; Auvin, S.; Cornet, S.; Demerle-Pallardy, C.; Guilmard-Favre, C.; Marin, J.-G.; Pignol, B.; Gillard-Roubert, V.; Roussillot-Charnet, C.; Schulz, J.; Viossat, I.; Bigg, D.; Moncada, S. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 10824.
8. Lodyato, V. I.; Yurkova, I. L.; Sorokin, V. L.; Shadyro, O. I.; Dolgopalets, V. I.; Kisel, M. A. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1179.
9. **4a**: ^{1}H NMR (200 MHz, CD_3OD) δ 7.24 (d, $J = 1.9$ Hz, 1H), 6.94 (d, $J = 2.0$ Hz, 1H), 2.78 (br, 4H), 1.40 (s, 9H). 1.26 (s, 9H), MS m/z 321.25 (M^+), 303.25 $(M-H_2O)^+$, 221.20 ($C_{14}H_{23}NO^+$), mp 184 °C. **4b**: ^{1}H NMR (200 MHz, CD_3OD–$CDCl_3$) δ 7.24 (d, $J = 2.0$ Hz, 1H), 6.95 (d, $J = 2.0$ Hz, 1H), 2.54 (t, $J = 7.1$ Hz, 2H), 2.38 (t, $J = 7.0$ Hz, 2H), 1.76 (m, 4H), 1.39 (s, 9H), 1.26 (s, 9H), MS m/z 349.30 (M^+), 221.20 ($C_{14}H_{23}NO^+$), mp 175 °C. **4c**: ^{1}H NMR (200 MHz, CD_3OD–$CDCl_3$) δ 7.22 (d, $J = 2.1$ Hz, 1H), 6.94 (d, $J = 2.0$ Hz, 1H), 2.50 (t, $J = 7.1$ Hz, 2H), 2.32 (t, $J = 6.8$ Hz, 2H) 1.44 (m, 13H), 1.26 (s, 9H), MS m/z 377.40 (M^+), 221.20 ($C_{14}H_{23}NO^+$), mp 157 °C. **4d**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 10.00 (br, 1H), 8.52 (br, 1H), 7.42 (br, 1H), 7.22 (d, $J = 1.9$ Hz, 1H), 6.78 (d, $J = 2.1$ Hz, 1H), 2.34 (m, 4H), 1.70 (m, 4H), 1.42 (m, 17H), 1.26 (s, 9H), MS m/z 405.40 (M^+), 221.20 ($C_{14}H_{23}NO^+$), mp 146 °C. **4e**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 11.90 (br, 1H), 8.52 (br, 1H), 7.41 (br, 1H), 7.22 (d, $J = 2.0$ Hz, 1H), 6.78 (d, $J = 2.0$ Hz, 1H), 2.39 (m, 4H), 1.74 (m, 4H), 1.44 (s, 9H), 1.26 (m, 21H), MS m/z 433.40 (M^+), 221.20 ($C_{14}H_{23}NO^+$), mp 143 °C. **4f**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 11.00 (br, 1H), 8.50 (br, 1H), 7.42 (br, 1H), 7.22 (d, $J = 2.0$ Hz, 1H), 6.78 (d, $J = 2.1$ Hz, 1H), 2.42 (m, 4H), 1.74 (m, 4H), 1.44 (s, 9H), 1.26 (m, 25H), MS m/z 461.20 (M^+), 221.20 ($C_{14}H_{23}NO^+$), mp 151C. **5**: ^{1}H NMR (200 MHz, CD_3OD) δ 7.24 (d, $J = 1.9$ Hz 1H), 6.94 (d, $J = 1.9$ Hz 1H), 3.26 (s, 9H), 2.45 (t, $J = 7.1$ Hz, 2H), 1.56 (m, 4H), 1.40 (s, 9H), 1.26 (m, 21H), mp 96 °C.
10. Zav'yalov, S. I.; Kravchenko, N. E. *Izv. Akad. Nauk SSSR, Ser. Khim.* **1988**, 1696, in Russian.
11. Shadyro, O. I.; Yurkova, I. L.; Kisel, M. A. *Int. J. Radiat. Biol.* **2002**, *78*, 211. Instead of 0.15 NaCl solution 0.2 M phosphate buffer (pH 7.4) was used.
12. Chan, H. W. S.; Levett, G. *Lipids* **1977**, *12*, 99.
13. Buege, J. A.; Aust, S. D. *Meth. Enzymol.* **1978**, *52*, 302.
14. Pomana College Medicinal Chemistry Project, Claremont, CA, USA.