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Short communication

Novel keto-enamine Schiffs bases from 7-hydroxy-4-methyl-2-oxo-2*H*-benzo[h] chromene-8,10-dicarbaldehyde as potential antidyslipidemic and antioxidant agents ☆

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Abstract

A series of Schiff bases have been synthesized from dicarbaldehyde of benzocoumarin, in which the reactions were regioselective and the products existed in the keto-enamine form, in which the aromaticity of the relevant ring was disrupted. The compounds were evaluated in vitro for their antioxidant and in vivo for their antidyslipidemic activity for the first time. Compounds **3** and **7** possess significant lipid lowering and antioxidant activity.

Keywords: Keto-enamine Schiff bases; Antioxidant; Lipid lowering activity

1. Introduction

Coumarins are one of the most important classes of fluorescent molecules and they are found to possess versatile biological activities [1]. They have been used to treat such diverse ailments as cancer, burns, brucellosis rheumatic [2] and cardiovascular diseases. Various coumarin related derivatives are recognized as inhibitors of the lipoxygenase and cyclooxygenase pathways of arachidonate metabolism [3–5] but also of neutrophile dependent superoxide anion generation.

Many coumarin derivatives have special ability to scavenge reactive oxygen species (ROS) free radicals, such as hydroxyl radicals, superoxide radicals or hypochlorous acid and to influence processes involving free radical injury [6,7]. Coumarins have been evaluated in vitro for their inhibitory activity toward bovine alfa-chymotrypsin, human leukocyte elastase [8–10] and thrombin plasmin and tissue plasminogen activator. Many coumarins were found to inhibit lipid peroxidation and to scavenge hydroxyl radicals and superoxide anion [11].

Recently, Madhavan et al. [12] have shown that coumarin derivatives of heterocyclic compounds can act as lipid lowering agents. Oxidative stress has recently been implicated in the pathogenesis of various diseases such as diabetes and CAD. Consequently, the potential therapeutic or preventive effects of antioxidative agents have been mentioned.

Thus, we found evaluating the Schiff base derivatives for their antioxidant behaviour and their antidyslipidemic activity interesting. The structure of our derivatives in addition to the biological active coumarin nucleus, possesses amine moiety and extended conjugation that is responsible for its highly fluorescent and antioxidant nature.

2. Chemistry

In continuation of our drug discovery programme we had synthesized 4-substituted coumarin derivatives.

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Our synthetic strategy to prepare 4-substituted coumarin started with widely used Pechmann reaction [13] between naphthalene 1,5-diol **1**, and beta-keto ester to give 7-hydroxy-4-methyl-benzo(h)chromene-2-one **2**. The resultant coumarin **2** was subjected to Duff reaction [14] to give 7-hydroxyl-4-methyl-2-oxo-2*H*-benzo(h)chromene-8,10 dicarbaldehyde **3**. As the effective role of azomethine linkage in certain biological reactions is well documented [15], we further wanted to introduce a nitrogenous side chain to improve its pharmacological activity. The dialdehyde **3** was condensed with various primary amines to give the respective Schiffs bases **4** (Scheme 1). These bases are converted into enamines because of extended conjugation in this system that stabilize them and that is the driving force for overcoming aromatic stabilization. The synthesis and detailed NMR characterization of the derivatives were given in our previous publication [16].

In some cases the purification of the compounds was very difficult. Some derivatives were recrystalized by absolute ethanol. The structures of the compounds were confirmed by spectral analysis (Table 1).

3. Biological activity

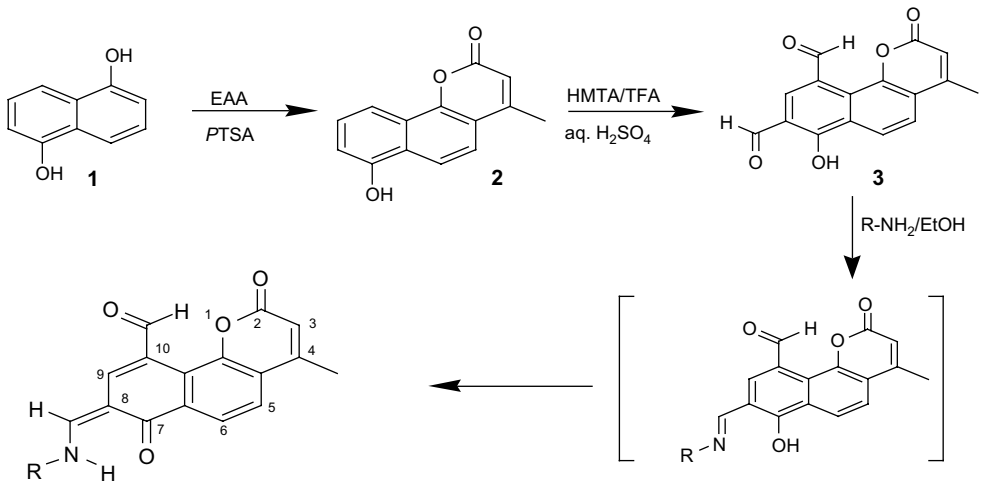
The present study has been undertaken to evaluate the antidyslipidemic activity of benzocoumarin derivatives of Schiff bases in triton model. The antioxidant activity of these derivatives was also evaluated by generating free radicals in vitro in the absence and presence of these derivatives.

3.1. Animals used

Rats (Charles Foster strain, male, adult, body weight 200–225 g) were kept in a room with controlled temperature (25–26 °C), humidity (60–80%) and12/12 h light/dark cycle (light on from 8.00 A.M. to 8.00 P.M.) under hygienic conditions. Animals, which were acclimatized for one week before starting the experiment, had free access to the normal diet and water.

3.2. Lipid lowering activity

Rats were divided into ten groups control, triton induced, triton plus 2, 3, 4, 5, 7, 8, 10, 11 and Gemfibrozil (100 mg/kg) treated groups containing six rats in each group. In this experiment of 18 h, hyperlipidemia was developed by administration of triton WR-1339 (Sigma chemical company, St. Louis, MO, USA) at a dose of 400 mg/kg. b.w. intraperitoneally to animals of all the groups except the control. These derivatives were macerated with gum acacia (0.2% w/v), suspended in water and fed simultaneously with triton at a dose of 100 mg/kg po to the animals of treated group and the diet being withdrawn. Animals of control and triton group without treatment with coumarin compounds were given same amount of gum acacia suspension (vehicle). After 18 h of treatment the animals were anaesthetized with thiopentone solution (50 mg/kg b.w.) prepared in normal saline and then 1.0 ml blood was withdrawn from retro-orbital sinus using glass capillary in EDTA coated Eppendorf tube (3.0 mg/ml blood). The blood was centrifuged (at 2500g) at 4 °C for 10 min and plasma was separated. Plasma was diluted with normal saline (ratio of 1:3) and used for analysis of total cholesterol (Tc), triglycerides (Tg) and phospholipids (Pl) by standard enzymatic methods [17] using Beckmann auto-analyzer and standard kits purchased from Beckmann Coulter International, USA.



Scheme 1.

Table 1
Spectral data of compounds (**2–11**)

Compound	ESIMS (M^{+1})	IR (ν_{max}) (KBr, cm^{-1})				^{1}H NMR (300 MHz)
		ν_{NH}	ν_{OH}	$\nu_{H-C=O}$	$\nu_{O-C=O}$	
2	227	–	3176	–	1677	(DMSO-d_6) δ 10.59 (s, 1H, –OH, exchangeable), 8.02 (d, $J = 12.0$ Hz, 1H, C6–H), 7.76 (d, $J = 9.0$ Hz, 1H, C10–H), 7.64 (d, $J = 9.0$ Hz, 1H, C5–H), 7.48 (t, $J = 9.0$ Hz, 1H, C9–H), 7.05 (d, $J = 6.0$ Hz, 1H, C8–H), 6.43 (s, 1H, C3–H), 2.47 (s, 3H, $-CH_3$).
3	283	–	3398	1727	1631	(DMSO-d_6) δ 10.96 (s, 1H, –CHO), 10.32 (s, 1H, –CHO), 8.33–8.29 (m, 2H, C6, C8–H), 7.95 (d, $J = 9.0$ Hz, 1H, C5–H), 6.65 (s, 1H, C3–H), 2.52 (s, 3H, $-CH_3$).
4	338	3432	–	1737	1595	($CDCl_3$) δ 13.83 (s, 1H, –NH, exchangeable), 11.14 (s, 1H, –CHO), 8.43 (d, $J = 9.0$ Hz, 1H, C6–H), 8.15–8.11 (m, 2H, C9–H, –NH–*CH*), 7.64 (d, $J = 9.0$ Hz, 1H, C5–H), 6.45 (s, 1H, C3–H), 2.67 (s, 3H, $-CH_3$), 1.52 (s, 9H, *t*-butyl).
5	324	3434	–	1737	1597	($CDCl_3$) δ 13.56 (s, 1H, –NH, exchangeable), 11.14 (s, 1H, –CHO), 8.44 (d, $J = 6.0$ Hz, 1H, C6–H), 8.12–8.06 (m, 2H, C9–H, –NH–*CH*), 7.66 (d, $J = 9.0$ Hz, 1H, C5–H), 6.45 (s, 1H, C3–H), 4.0–3.91 (m, 1H, –NH–*CH*), 2.67 (s, 3H, $-CH_3$), 1.52 (d, $J = 6.0$ Hz, 6H, isopropyl-CH_3).
6	296	3407	–	1729	1658	($CDCl_3$) δ 13.52 (s, 1H, –NH, exchangeable), 11.17 (s, 1H, –CHO), 8.48 (d, $J = 6.0$ Hz, 1H, C6–H), 8.12 (s, 1H, C9–H), 8.03 (d, $J = 12.0$ Hz, 1H, HN–*CH*=), 7.68 (d, $J = 9.0$ Hz, 1H, C5–H), 6.46 (s, 1H, C3–H), 3.50 (d, $J = 4.0$ Hz, 3H, $-HN-CH_3$), 2.58 (s, 3H, $-CH_3$).
7	380	3429	–	1719	1640	($CDCl_3$) δ 13.50 (s, 1H, –NH, exchangeable), 11.15 (s, 1H, –CHO), 8.47 (d, $J = 9.0$ Hz, 1H, C6–H), 8.13 (s, 1H, C9–H), 8.05 (d, $J = 12.0$ Hz, 1H, HN–*CH*=), 7.65 (d, $J = 9.0$ Hz, 1H, C5–H,), 6.45 (s, 1H, C3–H), 3.73–3.67 (m, 2H, $-HN-CH_2$), 2.51 (s, 3H, $-CH_3$), 1.78–0.91 (m, 13H, heptyl).
8	310	3437	–	1731	1643	($CDCl_3$) δ 13.54 (s, 1H, –NH, exchangeable), 11.16 (s, 1H, –CHO), 8.47 (d, $J = 9.0$ Hz, 1H, C6–H), 8.12 (s, 1H, C9–H), 8.04 (d, $J = 12.0$ Hz, 1H, HN–*CH*=), 7.66 (d, $J = 9.0$ Hz, 1H, C5–H), 6.46 (s, 1H, C3–H), 3.75 (m, 2H, $HN-CH_2$), 2.58 (s, 3H, $-CH_3$), 1.53 (t, 3H, CH_2-CH_3).
9	324	3410	–	1728	1595	($CDCl_3$) δ 13.52 (s, 1H, –NH, exchangeable), 11.13 (s, 1H, –CHO), 8.44 (d, $J = 6.0$ Hz, 1H, C6–H), 8.10 (s, 1H, C9–H), 8.05 (d, $J = 12.0$ Hz, 1H, HN–*CH*=), 7.65 (d, $J = 6.0$ Hz, 1H, C5–H), 6.45 (s, 1H, C3–H), 3.66–3.64 (m, 2H, $HN-CH_2$), 2.62 (s, 3H, $-CH_3$), 1.92 (m, 2H, $-CH_2-CH_2-CH_3$), 1.08 (t, 3H, $-CH_2-CH_2-CH_3$).
10	338	3422	–	1722	1590	($CDCl_3$) δ 13.44 (s, 1H, –NH, exchangeable), 11.05 (s, 1H, –CHO), 8.32 (d, $J = 9.0$ Hz, 1H, C6–H), 8.05–7.98 (m, 2H, C9–H, –HN–*CH*=), 7.63 (d, $J = 8.6$ Hz, 1H, C5–H), 6.41 (s, 1H, C3–H), 3.68–3.66 (br m, 2H, $HN-CH_2$), 2.53 (s, 3H, $-CH_3$), 1.85–1.75 (m, 2H, $-CH_2-CH_2-CH_2-CH_3$), 1.61–1.44 (m, 2H, $-CH_2-CH_2-CH_2-CH_3$), 1.06 (t, 3H, $-CH_2-CH_2-CH_2-CH_3$).
11	395	3427	–	1726	1590	($CDCl_3$) δ 13.39 (s, 1H, –NH, exchangeable), 11.13 (s, 1H, –CHO) 8.44 (d, $J = 9.0$ Hz, 1H, C6–H), 8.10–8.05 (m, 2H, C9–H, –HN–*CH*=), 7.64 (d, $J = 8.6$ Hz, 1H, C5–H), 6.45 (s, 1H, C3–H), 3.79–3.74 (m, 10H, $-HNCH_2-CH_2$, morpholine ring), 3.50–3.44 (m, 2H, $-HNCH_2-CH_2$), 2.58 (s, 3H, $-CH_3$).

3.3. Antioxidant activity (generation of free radicals)

Super oxide anions (O^{-2}) were generated enzymatically [18] by xanthine (160 mM), xanthine oxidase (0.04 U) and nitroblue tetrazolium (320 μM) in absence or presence of compounds (100 μg/ml) in 100 mM phosphate buffer (pH 8.2). Fractions were sonicated well in phosphate buffer before use. The reaction mixtures were incubated at 37 °C and after 30 min the reaction was stopped by adding 0.5 ml glacial acetic acid. The amount of formazone formed was measured at 560 nm on a spectrophotometer. Percentage inhibition was calculated taking absorption coefficient of formazone as $7.2 \times 10^3\ M^{-1}\ cm^{-1}$. In another set of experiment, an effect of compounds on generation of hydroxyl radicals (OH^-) was also studied by non-enzymic reactants [19]. Briefly OH^- were generated in a non-enzymic system comprised of deoxy ribose (2.8 mM), $FeSO_4 \cdot 7H_2O$ (2 mM), sodium ascorbate (2.0 mM) and H_2O_2 (2.8 mM) in 50 mM KH_2PO_4 buffer, pH 7.4 to a final volume of 2.5 ml. The above reaction mixtures in the absence or presence of compounds (100 μg/ml) were incubated at 37 °C for 90 min. Reference samples and reagent blanks were also run simultaneously. Malondialdehyde (MDA) content in both experimental and reference samples were estimated spectrophotometrically by thiobarbituric acid method as mentioned above [20].

3.4. Statistical evaluation

Data were analyzed using Student's t-test. The hyperlipidemic groups were compared with control drug treated groups. Similarly the generations of oxygen free radicals with different benzocoumarin derivatives were compared with that of their formation without compounds. $P < 0.05$ was considered to be significant.

4. Results and discussion

4.1. Effect of benzocoumarin derivatives on hyperlipidemia

Administration of Triton WR-1339 in rats induced marked hyperlipidemia as evidenced by increase in the

plasma level of Tc (3-fold) Pl (2.76-fold) and Tg (2.87-fold) as compared to control. Treatment of hyperlipidemic rats with benzocoumarin derivatives at the dose of 100 mg/kg p o reversed the plasma levels of lipid with varying extents. Compounds **3**, **7** and **11**, showed significant decrease in plasma levels of Tc, Pl and Tg by 23, 25, 21%; 24, 20, 22% and 20, 21, 25%, respectively, while compounds **2**, **4**, **5**, **8**, and **10** showed mild lipid lowering activity. These data compared with standard drug Gemfibrozil at the similar dose of 100 mg/kg showed decrease in plasma levels of Tc, Pl, Tg by 33, 32, 34%, respectively (Fig. 1).

4.2. Effect of benzocoumarin derivatives on oxygen free radical generation in vitro

The scavenging potential of coumarin derivatives at 100 μg/ml against formation of O_2^- and OH^- in non-enzymic systems was studied (Fig. 2). Further, the effect of compounds on lipid peroxidation in microsomes was also studied. Compounds **3** and **7** showed significant decrease in superoxide anions inhibition by 72 and 45%, respectively, and hydroxyl radicals inhibition by 52 and 49%, respectively. However, compounds **3**, **7**, **10** and **11** showed 27, 24, 37, 31% inhibition in microsomal lipid peroxidation, respectively. The standard drug Alloperinol at 20 μg/ml showed 90% inhibition in superoxide anions. Manitol and α-Tocopherol at the same dose of 100 μg/ml showed 48 and 45% inhibition of hydroxyl ions and microsomal lipid peroxidation, respectively. However, compounds **6** and **9** did not show any significant activity. Compounds **3** and **7** showed good activity in both lipid lowering and antioxidant experiments, compound **3** having aromatic dialdehyde with free hydroxyl at position 7 and in case of compound **7** (R = heptyl amine)

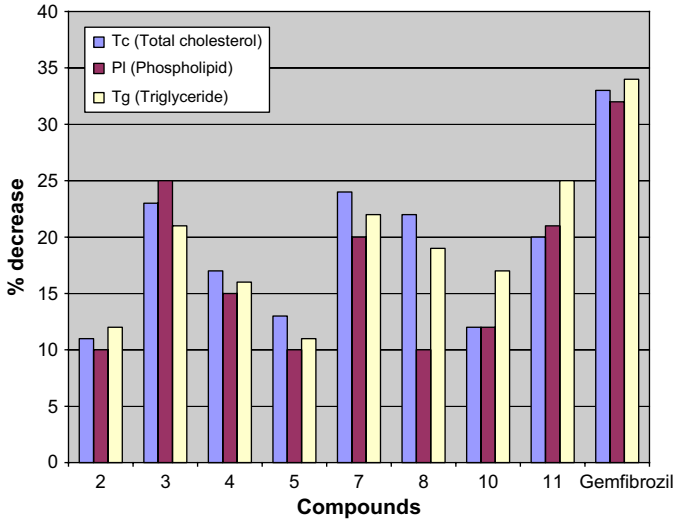


Fig. 1. The lipid lowering activity of different benzocoumarin derivatives (100 mg/kg) in triton treated hyperlipidemic rats is shown. Triton treated group is compared with control and drug treated group is compared with triton group (units – mg/dl).

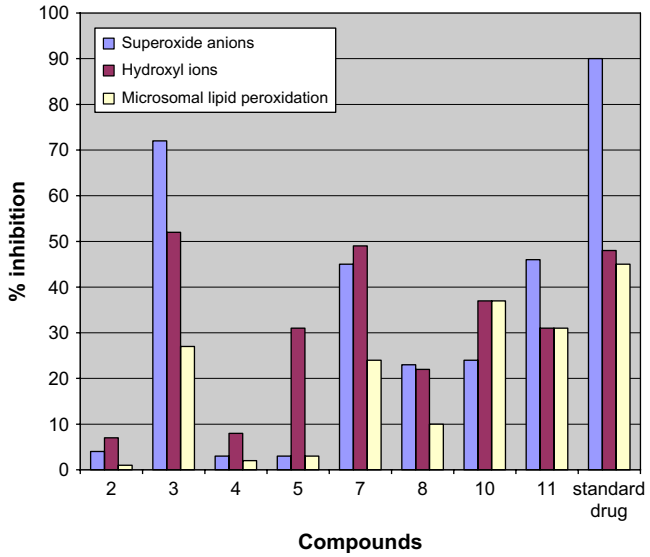


Fig. 2. The effect of benzocoumarin derivatives (100 μg/ml) on superoxide ion (*n* mol formazone formed/min), hydroxyl ion (*n* mol MDA formed/h) and lipid peroxidation in microsomes (*n* mol MDA formed/mg protein) is shown (standard drugs for superoxide anions – Alloperinol (20 μg/ml), hydroxyl ions – Manitol and for microsomal lipid peroxidation – α-Tocopherol (100 μg/ml) were used).

the significant activity could be attributed to optimum lipophilicity that might be playing an important role.

The involvement of hydroxyl free radicals (OH^-) has been found to be a major causative factor for peroxidative damage to lipoproteins which is responsible for inhibition and progression of atherosclerosis in hyperlipidemic subject [21]. Hyperlipidemia may also induce other abnormalities like oxidation of fatty acids, leading to the formation of ketone bodies as well as masking liver and muscle resistance to insulin which initiates the progress of diabetes in patients [22]. Furthermore, due to hyperglycemia, increase in non-enzymic glycosylation occurs, accompanied with glucose oxidation and these reactions being catalyzed by Cu^{+2} and Fe^{+2}, result in formation of O^{-2} and OH^- radicals which further accelerates the risk of cardiac diseases in dyslipidemic patients [23].

In order to overcome these ailments, a drug having multifold properties such as antioxidant, anti-diabetic and lipid lowering activities is in great demand. In our present study, we have investigated these properties in different derivatives of benzocoumarins. Both **3** and **7** caused significant decrease in the plasma level of lipid in triton models of hyperlipidemia. Triton WR-1339 acts as surfactant, suppresses the action of lipase and blocks the uptake of lipoproteins from the circulation by extrahepatic tissues resulting in an increase in the levels of circulating lipid [24]. These test samples inhibited cholesterol biosynthesis and potentiated the activity of lipolytic enzymes to early clearance of lipids from circulation in triton-induced hyperlipidemia. We have successfully used this model for evaluation of lipid lowering activity of benzocoumarin derivatives. To access the antioxidant activity of benzocoumarin derivatives, we have used in vitro model of non-enzymic and enzymic superoxide and hydroxyl radicals generation.

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References

[1] (a) M. Curini, G. Cravatto, F. Epifano, G. Giannone, Curr. Med. Chem. 13 (2006) 199–222;
(b) Z.Y. Yang, Y. Xia, P. Xia, A. Brossi, L.M. Cosentino, K.H. Lee, Bioorg. Med. Chem. Lett. 10 (2000) 1003–1005.
[2] R.D.H. Murray, I. Mendez, S.A. Brawn, The Natural Coumarins, Wiley, New York, 1982.
[3] T. Neichi, Y. Koshihara, S.I. Mutora, Biochim. Biophys. Acta 753 (1983) 130–132.
[4] Y. Kimura, H. Okuda, S. Arichi, K. Baba, M. Kowaza, Biochim. Biophys. Acta 834 (1985) 224–229.
[5] P.A. Craven, J. Pfanstiel, F.R. De Rubertis, J. Clin. Invest. 77 (1986) 850–859.
[6] R.A. Larson, Phytochemistry 27 (1988) 969–978.
[7] A. Mora, M. Paya, J.L. Rios, M.J. Alcaraz, Biochem. Pharmacol. 40 (1990) 793–798.
[8] C. Doucet, L. Pochet, N. Theierry, B. Pirotte, J. Delarge, M. Rebound-Ravaux, J. Med. Chem. 42 (1999) 4161–4171.
[9] D. Egan, E. O'Kennedy, E. Moran, D. Cox, E. Prosser, R.D. Thornes, Drug. Metab. Rev. 22 (1990) 503–529.
[10] D.N. Nicolaides, K.C. Fylaktakidou, K.E. Litinas, D. Hadjipavlou-Litina, J. Het. Chem. 33 (1996) 967–971.
[11] M. Paya, B. Halliwell, J.R.S. Hoult, Biochem. Phamacol. 44 (1992) 205–214.
[12] G.R. Madhavan, V. Balaraju, B. Mallesham, R. Chakrabarti, V.B. Lohray, Bioorg. Med. Chem. Lett. 13 (2003) 2547–2551.
[13] (a) H. Pechmann, C. Duisberg, Chem. Ber. 17 (1884) 929;
(b) V. Hasan, S. Abbas, Tetrahedron Lett. 46 (2005) 3501–3503;
(c) S. Teizo, T. Koichi, Chem. Lett. (2001) 110–111.
[14] F.L. Jay, N.J. Eric, G. Yun, H. Yaping, N. Xiaoyi, M.Z. Charles, J. Org. Chem. 59 (1994) 1939–1942.
[15] D. Hadjipavlou-Litina, A. Geronikaki, Drug Des. Discov. 15 (1998) 199–206.
[16] K.V. Sashidhara, J.N. Rosaiah, T. Narender, Tetrahedron Lett. 48 (2007) 1699–1702.
[17] (a) R. Deeg, J. Ziegehorn, Clin. Chem. 29 (1983) 1798–1802;
(b) G. Buccolo, H. David, Clin. Chem. 19 (1973) 476–482;
(c) D.B. Zilversmit, A.K. Davis, B.S. Memphis, J.L. Tenn, Clin. Med 35 (1950) 155–160.
[18] A. Bindoli, M. Valente, L. Cavallini, Pharmacol. Res. Commun. 17 (1985) 831–839.
[19] B. Halliwell, J.M.C. Gutteridge, O. Arouma, Anal. Biochem. 165 (1987) 215–219.
[20] H. Okhawa, N. Ohishi, K. Yagi, Anal. Biochem. 95 (1978) 351–358.
[21] S. Parthasarathy, D. Steinbert, J.L. Swiztum, Annu. Rev. Med. 43 (1992) 219–225.
[22] C.D.A. Stehouwer, J. Lambert, A.J.M. Donker, V.W.M. Van Hinsbergh, Cardiovasc. Res. 34 (1997) 55–68.
[23] T. Asahina, A. Kashiwagi, Y. Nishio, Diabetes 44 (1995) 520–526.
[24] P.E. Schurr, J.R. Schultz, T.M. Parkinson, Lipids 7 (1972) 68–74.