Bioorganic & Medicinal Chemistry Letters 21 (2011) 4480–4484

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and anti-inflammatory activity of novel biscoumarin–chalcone hybrids ☆

Koneni V. Sashidhara [a,*], Manoj Kumar [a], Ram K. Modukuri [a], Ravi Sonkar [b], Gitika Bhatia [b], A. K. Khanna [b], Shivika Rai [c], Rakesh Shukla [c]

[a] Medicinal and Process Chemistry Division, Central Drug Research Institute (CSIR-CDRI), Lucknow 226 001, India
[b] Biochemistry Division, Central Drug Research Institute (CSIR-CDRI), Lucknow 226 001, India
[c] Pharmacology Division, Central Drug Research Institute (CSIR-CDRI), Lucknow 226 001, India

ARTICLE INFO

Article history:
Received 15 March 2011
Revised 18 May 2011
Accepted 1 June 2011
Available online 12 June 2011

Keywords:
Synthesis
Biscoumarin–chalcone
Anti-inflammatory
TNF-α
Antioxidant

ABSTRACT

A series of synthesized novel biscoumarin–chalcone hybrids were evaluated for their anti-inflammatory and antioxidant activity. The tested compounds significantly inhibit the carrageenin induced paw oedema in albino rats and also exhibit important scavenging activities. These compounds thus constitute an interesting template for the design of new therapeutic tools against inflammation.

Inflammation is a self-protective response of body, which induces physiological adaptations to reduce tissue damage and to eliminate the pathogenic infections. It is a usual symptom covering different pathologies and causes a large number of diseases, amongst this, some commonest are rheumatoid arthritis (RA), inflammatory bowel disease, psoriasis and multiple sclerosis.[1,2] Cytokines have been recognised as necessary components in acute and chronic inflammatory processes. TNF-α is a major mediator of chronic inflammation and it can stimulate secretion of cytokines such as IL-1, IL-6, and IL-10.[3] Additionally, TNF-α inhibitors can be used to cure many diseases like rheumatoid arthritis, Alzheimer's disease, tumor, and obesity.[4] Most of the marketed TNF-α inhibitors are monoclonal antibodies (Adalimumab, Infliximab, Cimzia and Golimumab) that exhibit severe side effects. Therefore, there is a constant need for small molecules that selectively inhibit TNF-α.

Oxidative stress has been associated with the inflammation process. Reactive oxygen species like superoxide radical anion, hydrogen peroxide, and hydroxyl radical, are produced during the inflammation process by macrophages.[5] Thus, the discovery of molecules which combine anti-inflammatory and antioxidant activities may lead to the development of drugs with an improved therapeutic index. This has already been established for a number of commercially available nonsteroidal anti-inflammatory drugs (NSAIDs), which simultaneously possess radical scavenging properties.[6]

Coumarin and chalcone are important class of compounds having versatile biological activities.[7,8] Both of these pharmacophores are well known for their antioxidant and anti-inflammatory potential.[9] The coumarin nucleus incorporates the styryl carbonyl moiety known to possess appreciable anti-inflammatory activity while various chalcones (like naturally occurring butein, sappanchalcone and licochalcones) are known to exhibit potent scavenging activity (Fig. 1).[10–13] The combination of the distinct pharmacophores of two different biologically active compounds in the same structure (medicinal chemistry hybridization), has been previously reported in literature and is highly likely to lead to hybrid compounds with significant activity.[14] On the basis of these observations, we wanted to design and synthesize molecules which include both coumarin and chalcone pharmacophore in one frame. In continuation of our efforts to explore the chemical diversity space around coumarin scaffold, we have synthesized diverse class of coumarin compounds with good pharmacological profile.[15] In this Letter, we report here our attempt on antioxidant and anti-inflammatory evaluation of biscoumarin derivatives. The general synthetic strategy was employed to prepare biscoumarin–chalcone hybrid is based on methodology perfected in our lab.[16] The synthetic route used to synthesize title compounds is outlined in

☆ Part XI in the series, 'Advances in drug design and discovery'. CDRI publication no. 8075.

* Corresponding author. Tel.: +91 9919317940; fax: +91 522 2623405.
E-mail addresses: sashidhar123@gmail.com, kv_sashidhara@cdri.res.in (K.V. Sashidhara).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.06.002

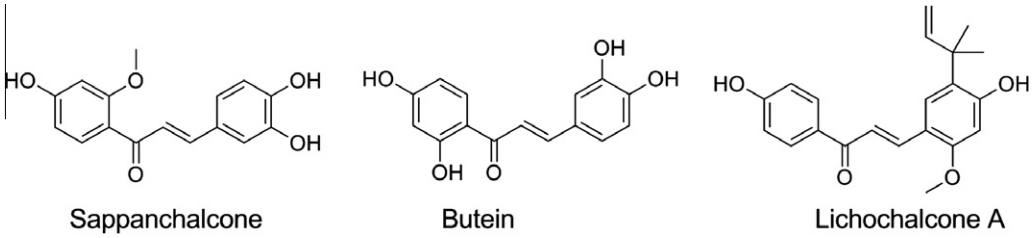
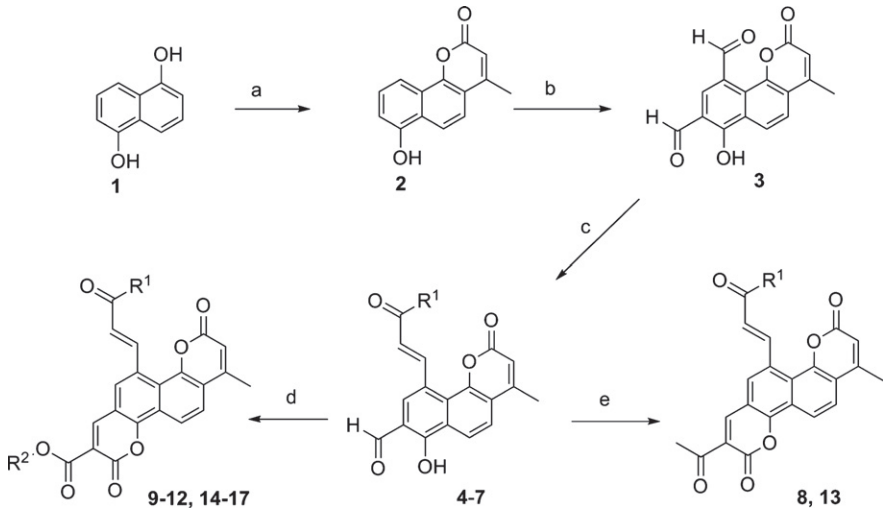


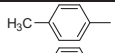
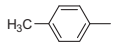
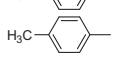
Figure 1. Few naturally occurring anti-inflammatory chalcone.

Scheme 1. Thus, the Pechmann reaction on 1,5-dihydroxynaphthelene (**1**), furnished 7-hydroxy-4-methylbenzo(h)chromene-2-one (**2**) which on the Duff formylation in the presence of hexamethylenetetraamine (HMTA) and TFA at 120 °C gave dicarbaldehyde (**3**), which on coupling with different appropriate acetophenones in refluxing dioxane, in the presence of a catalytic amount of concd HCl afforded regioselective *para*-condensed chalcones (**4–7**) in good yields. These chalcone derivatives on subsequent Knoevenagel-type condensation with different active methylene compounds furnished biscoumarin–chalcone hybrids in high yields (**8–17**). It was confirmed that the synthesized chalcones were in *trans*-configuration, as evidenced by their coupling constant. Structures of the compounds were substantiated by ^{1}H NMR, ^{13}C NMR, 2D NMR, Mass spectrometry and IR spectroscopy. The purity of these compounds was ascertained by TLC and spectral analysis (Supplementary data).[17]

Anti-inflammatory activity. In the present study, we carried out experiments to investigate the anti-inflammatory activity[18] of biscoumarin–chalcone hybrids (**8–17**) in carrageenin-induced paw oedema in albino rats. Oedema in one of the hind paws was induced by injection of carrageenin solution (0.1 ml of 1%) into planter aponeurosis. The volume of the paw was measured plethysmographically immediately after and 3 h after the injection of the irritant. The difference in volume gave the amount of oedema developed. Percent inhibition of the oedema between the control group and compound-treated groups was calculated and compared with the group receiving a standard drug. Anti-inflammatory activity of compounds (**8–17**) screened is presented in Table 1. Out of all compounds evaluated three compounds showed significant activity. The Compounds **8**, **13** and **17** at 100 mg/kg po exhibited 29%, 26%, and 33% activity, respectively, while the reference drug, ibuprofen exhibited 59.5% protection at an equivalent dose. While the compounds **11**, **12** and **14** showed moderate activities. The in vivo examined compounds did not show any toxic effects in different doses studied and in almost all the cases, the animals survived (80–90%) and looked normal both macroscopically and by autopsy, demonstrating low general toxicity of the synthesized compounds.

Both coumarin and chalcones are well known inhibitors of TNF-α.[19,20] In the next step, the active compounds **8**, **13** and **17** were evaluated for their potential to inhibit TNF-α inhibition using whole blood assay.[21] All the said compounds showed TNF-α inhibi-



Compound	R^1	R^2	% Yield	Compound	R^1	R^2	% Yield
8			85	**13**			80
9		C_2H_5	81	**14**		C_2H_5	83
10		CH_3	79	**15**		CH_3	80
11		C_2H_5	86	**16**		C_2H_5	75
12		CH_3	78	**17**		CH_3	78

Scheme 1. Synthesis of novel biscoumarin–chalcone hybrids (**8–17**). Reagents and conditions: (a) ethyl acetoacetate, *p*-toluene sulphonic acid, 75 °C, 8 h; (b) (i) hexamethylenetetramine, trifluoroacetic acid, 120 °C, 4 h; (ii) aq H_2SO_4, 100 °C, 1 h; (c) R^1-$COCH_3$, concd HCl, dioxane, reflux, 5 h; (d) $CH_2(COOR^2)_2$, piperidine, EtOH, reflux, 30 min ; (e) ethyl acetoacetate , piperidine, EtOH, reflux, 30 min.

Table 1
In vivo anti-inflammatory activity of biscoumarin–chalcone hybrids (**8**–**17**) in carrageenin-induced paw oedema in albino rats and TNF-α inhibition

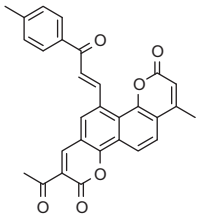
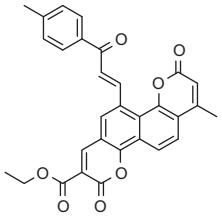
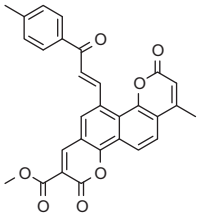
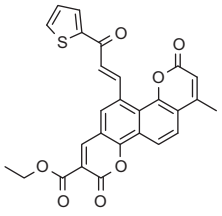
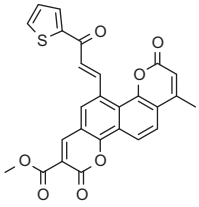
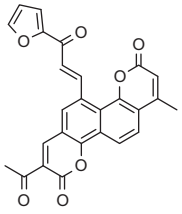
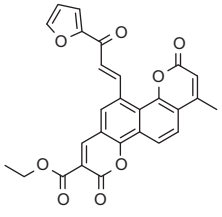
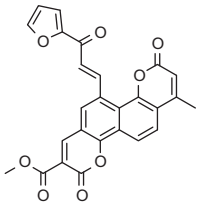
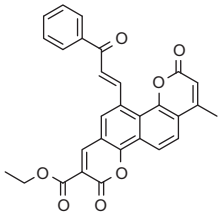
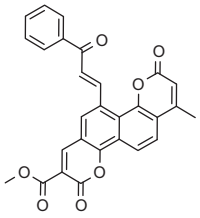
Compound	Structure	% Anti-inflammatory activity[a]	% Inhibition TNF-α	
8		29	Dose (μg/mL) 200 400	% Inhibition 33 47
9		NA		ND
10		NA		ND
11		8.77		ND
12		3.5		ND
13		26	Dose (μg/mL) 200 400	% Inhibition 25 43
14		13		ND

Table 1 (*continued*)

Compound	Structure	% Anti-inflammatory activity[a]	% Inhibition TNF-α	
15		NA	ND	
16		NA	ND	
17		33	Dose (μg/mL) 200 400	% Inhibition 04 21
Ibuprofen		59.5		
Pent(5 mM) + LPS (5 μg)			57	

NA—Not active.
ND—Not done.
[a] Dose 100 mg/kg po.

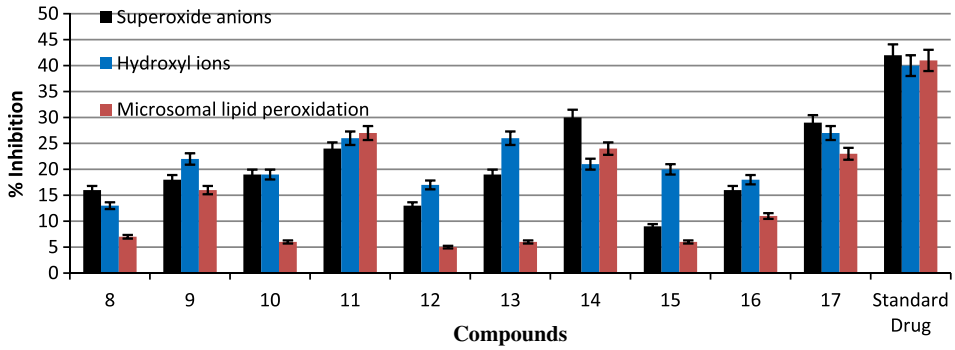


Figure 2. The effect of novel biscoumarin–chalcone (200 μg/mL) on superoxide ion (nmol formazone formed/min), hydroxyl ion (nmol MDA formed/h) and lipid peroxidation in microsomes (nmol MDA formed/mg protein) is shown (standard drugs for superoxide anions—Alloperinol (20 μg/mL), hydroxyl ions—Manitol and for microsomal lipid peroxidation-α-tocopherol (100 μg/mL) were used). Each value is mean ± SD of six values.

tion in dose dependent manner. Compounds **8**, **13** and **17** at a dose of 400 μg/mL showed 47%, 43% and 21% inhibition (Table 1).

Antioxidant activity. Antioxidant activities of compounds **8–17** were evaluated by generating free radicals in vitro in the absence and presence of these compounds.[22] The scavenging potential of biscoumarins–chalcones **8–17** at 200 μg/mL against formation of $O_2^{\cdot}$ and $^{\cdot}OH$ in non-enzymic systems were studied. Further, their effects on lipid peroxidation in microsomes were also studied (Fig. 2). Compounds **11**, **14** and **17** showed significant decrease in superoxide anions inhibition by 24%, 30% and 29%, hydroxyl radicals inhibition by 26%, 21% and 27% and microsomal lipid peroxidation inhibition by 27%, 24% and 23%, respectively (each value is mean ± SD of six values P <0.001). The standard drug Alloperinol at 20 μg/mL showed 42% inhibition in superoxide anions. Manitol and α-tocopherol at the dose of 100 μg/mL showed 40% and 41% inhibition of hydroxyl ions and microsomal lipid peroxidation, respectively. The scavenging potentials of other derivatives were modest. The propensity of radical formation and stabilization, ability of metal complexation and lipophilicity are important factors for the antioxidant activity. The wide variation in the free radical scavenging potential for the tested compounds may be due to the variation in the proton–electron transfer by the derivatives due to difference in their structures and stability.

In conclusion, a series of novel substituted biscoumarin–chalcone hybrids (**8–17**) have been synthesized in four steps starting from 1,5-dihydroxynaphthalene. Among the synthesized compounds, compound **17** was found to be the most active anti-inflammatory agent in addition to having potent antioxidant activity, suggesting that the anti-inflammatory property of the compound might be partly due to its radical scavenging activity. Thus, the compound **17** provides a useful starting point for the rational

design of anti-inflammatory agents with improved pharmacokinetics and pharmacodynamics properties.

Acknowledgments

Instrumentation facilities from SAIF, CDRI and financial support from CSIR, New Delhi, India to A.K., R.K.M. and S.R. are gratefully acknowledged. This is CSIR-CDRI contribution number 8075.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.06.002.

References and notes

1. Marc, F.; Brennan, F. M.; Brian, F.; Maini, R. N. *Curr. Dir. Autoimmun.* **2001**, *3*, 188.
2. Daniel, T.; Lars, K.; Eric, S. H.; Jochen, S. G.; Paul, T. P. *Pharmacol. Ther.* **2008**, *117*, 244.
3. Cottam, H. B.; Shih, H.; Tehrani, L. R.; Wasson, B.; Carson, D. A. *J. Med. Chem.* **1996**, *39*, 2.
4. (a) Ogata, H.; Hibi, T. *Curr. Pharm. Des.* **2003**, *14*, 1107; (b) Moller, D. R. *J. Intern. Med.* **2003**, *253*, 31; (c) Sack, M. *Pharmacol. Ther.* **2002**, *94*, 123; (d) Newton, R. C.; Decicco, C. P. *J. Med. Chem.* **1999**, *42*, 13.
5. Detsi, A.; Bouloumbasi, D.; Prousis, K. C.; Koufaki, M.; Athanasellis, G.; Melagraki, G.; Afantitis, A.; Igglessi-Markopoulou, O.; Kontogiorgis, C.; Hadjipavlou-Litina, D. J. *J. Med. Chem.* **2007**, *50*, 2450.
6. Orhan, H.; Sahin, G. *Exp. Toxicol. Pathol.* **2001**, *53*, 133.
7. Egan, D.; O'Kennedy, E.; Moran, E.; Cox, D.; Prosser, E.; Thornes, R. D. *Drug. Metab. Rev.* **1990**, *22*, 503.
8. Ni, L.; Meng, C. Q.; Sikorski, J. A. *Expert Opin. Ther. Pat.* **2004**, *14*, 1669.
9. Kontogiorgis, C. A.; Hadjipavlou, L. D. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 611.
10. Bandgar, B. P.; Gawande, S. S.; Bodade, R. G.; Totre, J. V.; Khobragade, C. N. *Bioorg. Med. Chem.* **2010**, *18*, 1364.
11. Kontogiorgis, C. A.; Hadjipavlou, L. D. *J. Med. Chem.* **2005**, *48*, 6400.
12. Nowakowska, Z. *Eur. J. Med. Chem.* **2007**, *42*, 125.
13. Okada, K.; Tamura, Y.; Yamamoto, M. Y.; Inoue, R.; Takagaki, K.; Takahashi, S.; Demizu, K.; Kajiyama, Y.; Kinoshita, T. *Chem. Pharm. Bull.* **1989**, *37*, 2528.
14. Lazar, C.; Kluczyk, A.; Kiyota, T.; Konishi, Y. *J. Med. Chem.* **2004**, *47*, 6973.
15. (a) Sashidhara, K. V.; Kumar, A.; Kumar, M.; Sonkar, R.; Bhatia, G.; Khanna, A. K. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4248; (b) Sashidhara, K. V.; Rosaiah, J. N.; Kumar, A.; Bhatia, G.; Khanna, A. K. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3065; (c) Sashidhara, K. V.; Rosaiah, J. N.; Bhatia, G.; Saxena, J. K. *Eur. J. Med. Chem.* **2008**, *43*, 2592; (d) Sashidhara, K. V.; Kumar, A.; Kumar, M.; Sarkar, J.; Sinha, S. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7205; (e) Sashidhara, K. V.; Kumar, A.; Kumar, M.; Srivastava, A.; Puri, A. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6504; (f) Sashidhara, K. V.; Kumar, A.; Chatterjee, M.; Rao, K. B.; Singh, S.; Verma, A. K.; Palit, G. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1937.
16. (a) Sashidhara, K. V.; Rosaiah, J. N.; Narender, T. *Tetrahedron Lett.* **2007**, *48*, 1699; (b) Sashidhara, K. V.; Rosaiah, J. N.; Kumar, M.; Gara, R. K.; Nayak, L. V.; Srivastava, K.; Bid, H. K.; Konwar, R. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7127.
17. *(E)-Methyl 7-methyl-3,9-dioxo-11-(3-oxo-3-phenylprop-1-enyl)-3,9-dihydrochromeno[8,7-h]chromene-2-carboxylate* (**17**). Yellow solid, yield: 78%; mp 137–138 °C; IR (KBr): 3015, 1726, 1548, 1217, 755 cm^{-1}; ^1H NMR (TFA d, 300 MHz) δ: 9.06 (s, 1H), 8.98 (d, J = 7.7 Hz, 1H), 8.59 (d, J = 4.3 Hz, 1H) 8.06 (br s, 4H), 7.62–7.49 (m, 4H), 6.71 (s, 1H), 4.05 (s, 3H), 2.65 (s, 3H): ^{13}C NMR (TFA d, 75 MHz) δ: 196.8, 164.6, 163.8, 157.5, 152.6, 151.6, 149.9, 148.4, 135.6, 134.5, 131.5, 128.9, 128.5, 126.8, 125.6, 124.6, 123.5, 123.4, 120.8, 119.8, 116.6, 115.2, 114.6, 53.0, 17.6: DEPT 135 (TFA d, 75 MHz) δ: 151.6, 148.4, 134.5, 128.9, 128.5, 126.8, 125.6, 123.4, 119.7, 114.6, 53.0, 17.6 : ESI-MS: (m/z): 467 $(M+H)^+$: HRMS m/z calcd for $C_{28}H_{18}O_7$ $(M+H)^+$ 467.1132, found 467.1103.
18. Winter, C. A.; Risley, E. A.; Nuss, G. W. *Proc. Soc. Exp. Biol. Med.* **1962**, *111*, 544.
19. Upadhyay, K.; Bavishi, A.; Thakrar, S.; Radadiya, A.; Vala, H.; Parekh, S.; Bhavsar, D.; Savant, M.; Parmar, M.; Adlakha, P.; Shah, A. *Bioorg. Med. Chem. Lett.* **2011**, doi:10.1016/j.bmcl.2011.02.016.
20. Bandgar, B. P.; Patil, S. A.; Korbad, B. L.; Nile, S. H.; Khobragade, C. N. *Eur. J. Med. Chem.* **2010**, *45*, 2629.
21. Hussain, R.; Mehmood, A.; Shahid, F.; Dojki, M.; Jamil, B.; Mehmood, H.; Dawood, G.; Dockrell, H. M. *J. Immunol. Methods* **2002**, *264*, 95.
22. *Antioxidant activity (generation of free radicals)*: Super oxide anions were generated enzymatically by xanthine (160 mM), xanthine oxidase (0.04 U), and nitroblue tetrazolium (320 μM) in absence or presence of compounds (**8**–**17**) (200 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 calculated spectrophotometrically. In another set of experiment effect of compounds on the generation of hydroxyl radical was also studied by non-enzymatic reactants. Briefly, OH^- were generated in a non-enzymatic system comprising 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 test compounds (200 μg/mL) were incubated at 37 °C for 90 min. The test compounds were also studied for their inhibitory action against microsomal lipid peroxidation in vitro by non-enzymatic inducer. Reference tubes and reagents blanks were also run simultaneously. Malondialdehyde (MDA) contents in both experimental and reference tubes were estimated spectrophotometrically by thiobarbituric acid as mentioned (Okhawa, H.; Ohishi, N.; Yagi, K. *Anal. Biochem.* **1978**, *95*, 351.). Alloprinol, Mannitol and α-tocopherol were used as standard drugs for superoxide, hydroxylations and microsomal lipid peroxidation. All experimental data were analyzed using Student's *t*-test. Oxidized LDL was compared with the test compounds treated oxidized LDL. The generation of oxygen free radicals was compared in the presence and absence of test compounds.