Khairia M. Youssef[a],
Magda A. El-Sherbeny[a],
Faiza S. El-Shafie[a],
Hassan A. Farag[a],
Omar A. Al-Deeb[a],
Sit Albanat A. Awadalla[b]

[a] Department of Medicinal Chemistry, College of Pharmacy and
[b] Department of Physiology, College of Medicine, King Saud University, Riyadh, Saudi Arabia

Synthesis of Curcumin Analogues as Potential Antioxidant, Cancer Chemopreventive Agents

New series of 3,5-bis(substituted benzylidene)-4-piperidones, 2,7-bis(substituted benzylidene)cycloheptanones, 1,5-bis(substituted phenyl)-1,4-pentadien-3-ones, 1,7-bis(substituted phenyl)-1,6-heptadien-3,5-diones, 1,1-bis(substituted cinnamoyl)- cyclopentanes, and 1,1-bis(substituted cinnamoyl)cyclohexanes have been synthesized and tested for their antioxidant activity. Among the tested compounds, compounds **II$_4$, II$_9$ II$_{10}$, II$_{11}$, V$_1$**, and **V$_4$** exhibited higher free radical scavenger activity with % inhibition values of 90.71, 91.24, 96.91, 94.26, 99.23, and 99.85 %, respectively. Moreover, compound **V$_1$** is the safest member toward peripheral multinuclear neutrophils (PMNs) with a % viability value of 91 %. Detailed synthesis, spectroscopic, and biological data are reported.

Keywords: Curcumin derivatives; Antioxidants; Free radical scavengers; Chemiluminescence test

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Introduction

Chemoprevention is the process of reversing, suppressing, or preventing carcinogenesis. A number of naturally-occuring and synthetic compounds of potential chemopreventive properties has been identified [1–4]. Some of these compounds appear to block activation of chemical carcinogens and prevent the initiation phase [5]. Lipid peroxidation is a term that refers to the oxidative degeneration of unsaturated lipids in cell membrane and other organized assemblies. Interest in biological lipid peroxidation has been intensified in recent years due to the growing awareness that lipid peroxidation may play an important role in aging and in a wide variety of pathological disorders such as arteriosclerosis, inflammatory disease, and cancer [6, 7]. It is a free radical-mediated chain reaction, in which lipid hydroperoxides are produced. Accumulation of lipid hydroperoxides in a membrane disrupts membrane functions, leads to decreased fluidity, increased leaking of ions, and damage to membrane proteins such as receptors and enzymes [8, 9]. The human body uses antioxidative mechanisms to quench this biochemical fire; among these are the enzymes superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSH-Px) [10]. Tissue stores of small molecules, such as α-tocopherol, reduced glutathione (GSH), and ascorbate, are also used. However, when these defenses are overwhelmed by pre-oxidants, toxic levels of oxyradicals may accumulate in tissues [11]. A number of naturally occuring compounds as β-carotene[12, 13], vitamin E [14], reduced glutathione [15, 16], green tea polyphenols [17], resveratrol [18], curcumin [19] and synthetic medicinals such as aspirin, sulindac, and piroxicam [20, 21] were investigated for their potential role in different types of animal model cancers as urinary bladder, lung tumorgenesis, and colon cancer. Curcumin has recently received considerable attention due to its pronounced anti-inflammatory [22], antibacterial [23], antihepatotoxic [24], and antioxidative properties [25]. The potent antioxidant activity of curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadien-3,5-dione, Figure 1) has currently received remarkable interest as it has a unique conjugated structure and shows a typical radical trapping ability as a chain-breaking antioxidant.

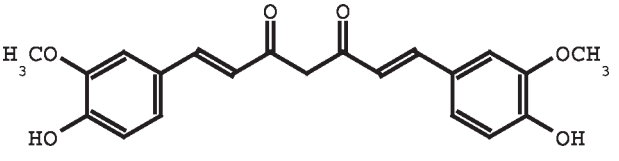


Figure 1.

The antioxidant mechanism of curcumin has attracted much attention. Generally, it has a dual effect in oxygen radical reactions, thus it can act as a scavenger of hydroxyl radicals or it catalyses the formation of hydroxyl radicals depending on the experimental conditions [26]. Curcumin is stable at a pH below 6.5, the instability of

Correspondence: Khairia M. Youssef, Department of Medicinal Chemistry, College of Pharmacy, King Saud University, P.O. Box 22452, Riyadh 11495, Saudi Arabia. Phone: +966-1-477 5464, Fax: +966-1-477 5464; e-mail: Khairia_m@yahoo.com

curcumin at a pH above 6.5 is attributed to the active methylene group, omitting the active methylene group leads to potent antioxidative compounds. In this work, a series of 3,5-bis(substituted benzylidene)-4-piperidones **II$_{1–16}$**, 2,7-bis(substituted benzylidene)cycloheptanones **III$_{1–5}$**, 1,5-bis(substituted phenyl)-1,4-pentadien-3-ones **IV$_{1–6}$**, 1,7-bis(substituted phenyl)-1,6-heptadien-3,5-diones **V$_{1–6}$**, 1,1-bis-(substituted cinnamoyl)cyclopentanes **VI$_{1–6}$**, and cyclohexanes **VII$_{1–6}$** are prepared and evaluated for their antioxidant activity compared with that of curcumin.

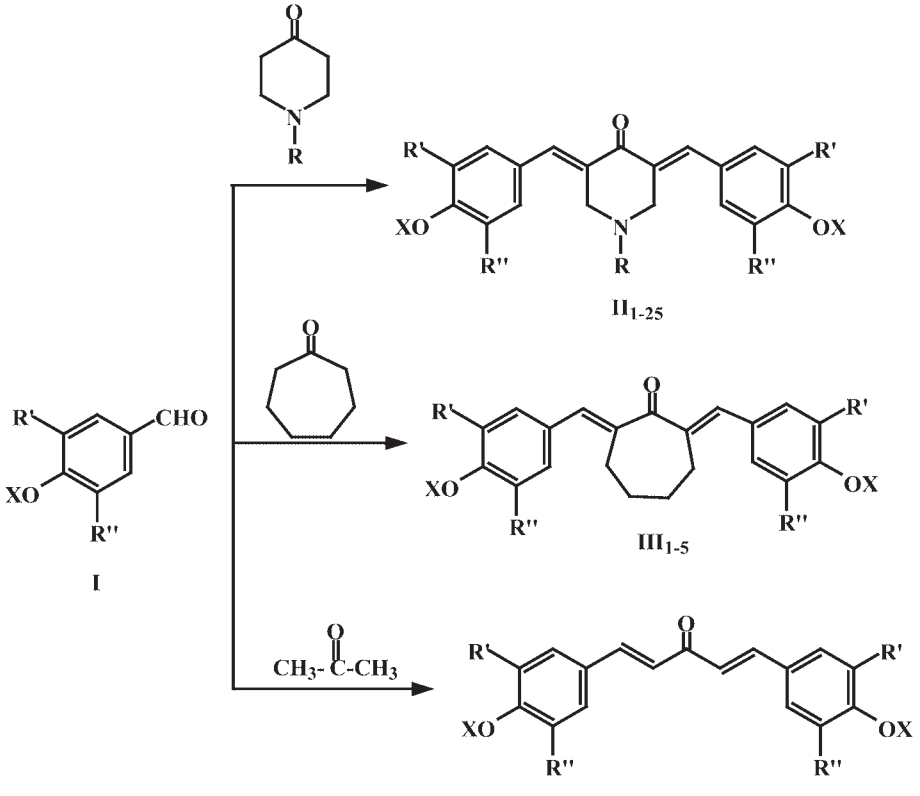
Chemistry

The designed target compounds are depicted in Schemes 1 and 2. 3,5-bis(substituted benzylidene)-N-alkyl-4-piperidones **II$_{1–16}$** were obtained through condensation of the appropriate 4-piperidone with a variety of aromatic aldehydes under acidic condition. Following the same reaction condition 2,7-bis(substituted benzylidene)-cycloheptanones **III$_{1–5}$** and 1,5-bis(substituted phenyl)-1,4-pentadien-3-ones **IV$_{1–6}$** were produced through condensation of the appropriate aldehyde with either 1-cycloheptanone or acetone, respectively (Scheme 1). The interaction of the appropriate aldehyde with acetyl acetone using alcoholic NaOH afforded the corresponding chalcone 1,7-bis(substituted phenyl)-1,6-heptadien-3,5-diones **V$_{1–6}$**. Upon treatment of compounds **V$_{1–6}$** with NaOEt, the corresponding sodium salts were obtained, which were subjected to cyclization using either 1,4-dibromobutane or 1,5-dibromopentane in ethanol to yield the required 1,1-bis(substituted cinnamoyl)cyclopentanes **VI$_{1–6}$** and 1,1-bis(substituted cinnamoyl)cyclohexanes **VII$_{1–6}$**, respectively (Scheme 2).

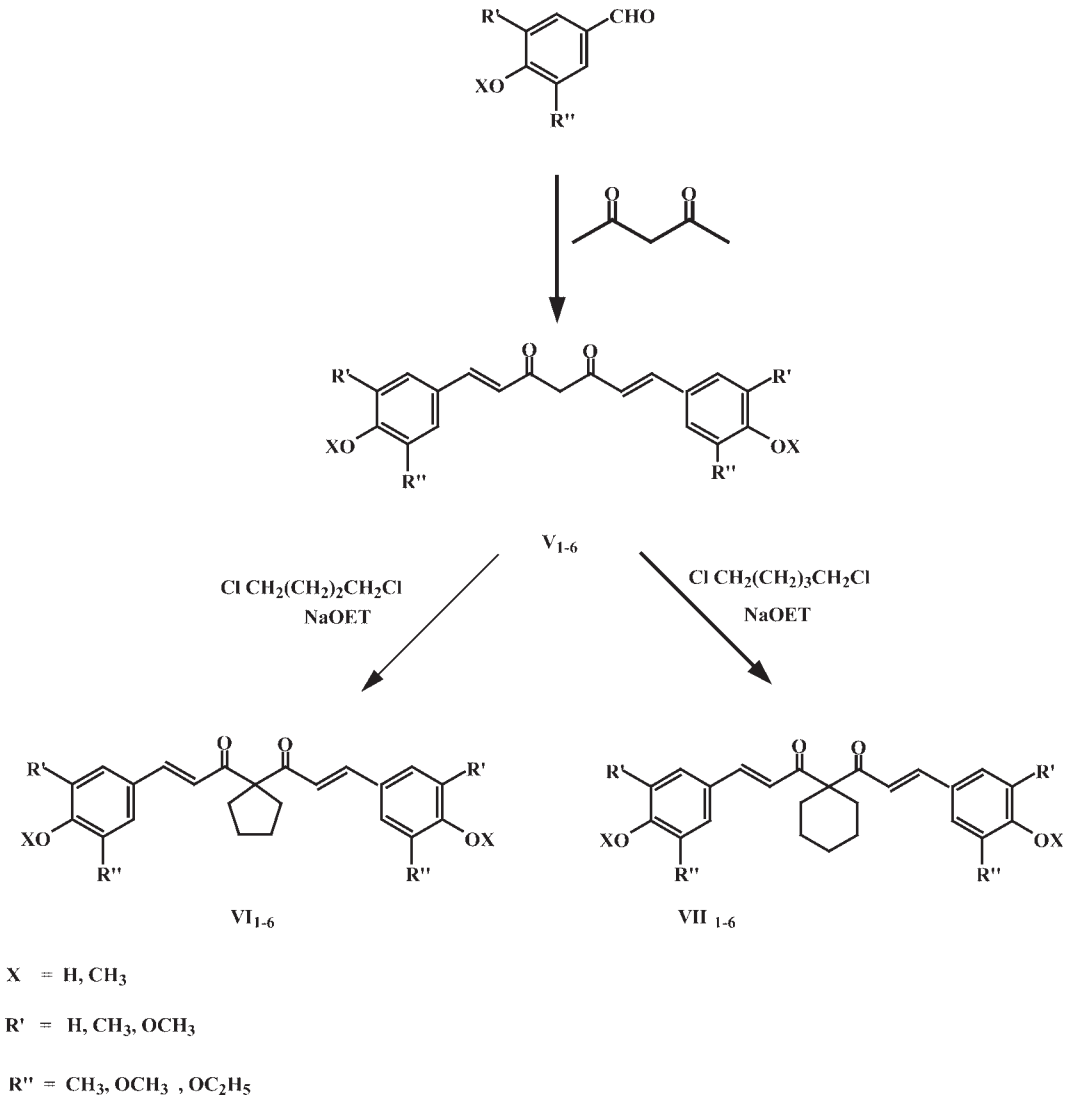
Biology

In vitro chromatographic determination of free radical scavenging activity (Diphenylpicrylhydrazyl free radical test)

The reactivity of the prepared compounds, as free radical scavengers, were tested by their action on the 2,2-diphenyl-1-picrylhydrazyl free radical. The test is simple



Scheme 1.



Scheme 2.

and was carried out in acetonitrile without any need of biological media. Diphenylpicrylhydrazyl absorbs strongly at 515 nm. The reaction was carried out under pseudo-first-order conditions using excess of the tested compounds compared to the reagent. The mixture was monitored for the decrease of absorption at 515 nm. Form the linear regression line of the absorbancy against time, the pseudo-first-order rate constant (K_{app}) and the corresponding half-life time ($t_{1/2}$) of the reaction were calculated. This reaction is valuable in studying the difference between the chemical and the *in vitro* biological testing [27].

Chemical determination for the free radical scavenging activity indicates that most of the tested compounds show higher activity compared to **curcumin** with K_{app} $min^{-1} \times 10^{-2}$ equal 3.30. In case of compounds **II**, high activities are observed with $\mathbf{II_3}$, $\mathbf{II_4}$, $\mathbf{II_6}$, $\mathbf{II_9}$, $\mathbf{II_{10}}$, $\mathbf{II_{11}}$, $\mathbf{II_{12}}$, $\mathbf{II_{13}}$, and $\mathbf{II_{15}}$ with K_{app} $min^{-1} \times 10^{-2}$ equal 7.30, 10.25, 4.40, 12.15, 16.32, 12.41, 9.32, 6.91, and 14.80, respectively. A comparison of activities within compounds **II** shows that the introduction of electron donating substituents at the ortho-position of the phenolic hydroxy group produces compounds with higher activity as shown by **II3** and **II4**, **II6 II1**, Kapp min-1—10-2 are 7.30, 10.25, 4.40 > 2.15, which is also true for $\mathbf{II_9}$, $\mathbf{II_{10}}$, $\mathbf{II_{11}}$, $\mathbf{II_{12}} > \mathbf{II_7}$ and $\mathbf{II_{15}} > \mathbf{II_{13}}$. Moreover, increasing the lipophilicity of the piperidone ring by substitution of methyl with either an ethyl or propyl moiety, potentiates the activity as shown by $\mathbf{II_{13}}$ and $\mathbf{II_7} > \mathbf{II_1}$, K_{app} $min^{-1} \times 10^{-2}$ are 6.91, 3.06 > 2.15. Replacement of the piperidone moiety with cycloheptanone affords compounds which still possesses high free radical scavenging activity, as shown by $\mathbf{III_1}$ and $\mathbf{III_5}$ with

Table 1. Reaction of Curcumin analogues with free radical.

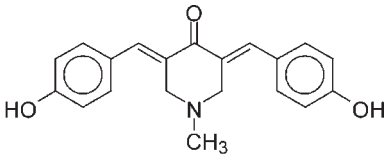
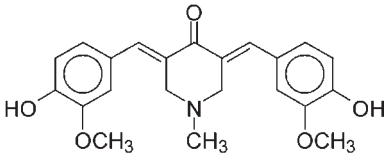
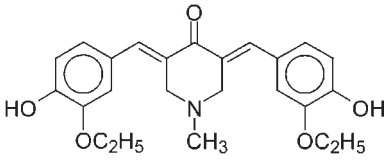
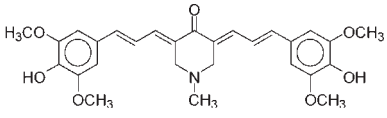
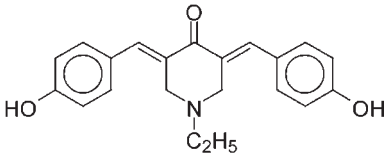
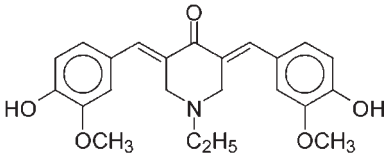
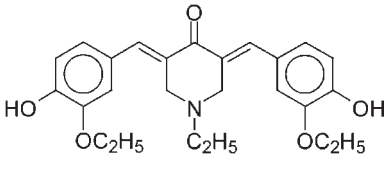
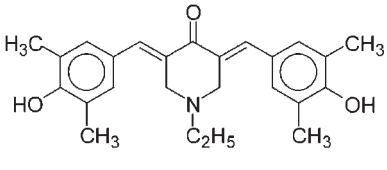
Compound	Structure	$Kmin^{-1} \times 10^{-2}$	r	T 1/2
II_1		2.15 ± 0.116	– 0.998	25.3
II_3		7.30 ± 0.11	– 0.999	19.5
II_4		10.25 ± 0.103	–0.996	17.5
II_6		4.40 ± 0.023	– 0.997	20.5
II_7		3.06 ± 0.057	– 0.995	12.9
II_9		12.15 ± 0.120	– 0.991	10.5
II_{10}		16.32 ± 0.035	–0.993	15.3
II_{11}		12.41 ± 0.032	–0.992	12.7

Table 1. (continued)

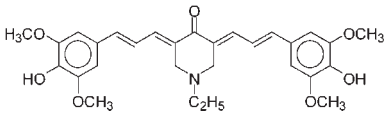
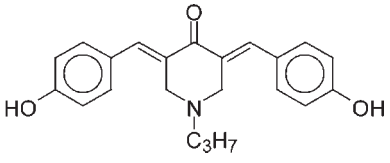
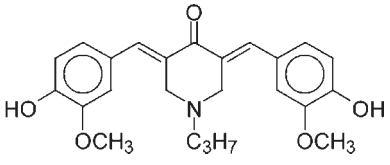
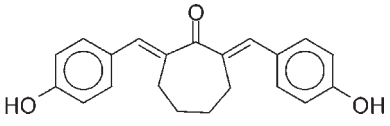
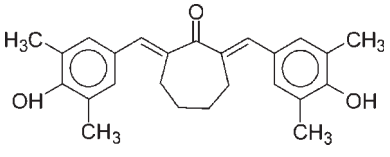
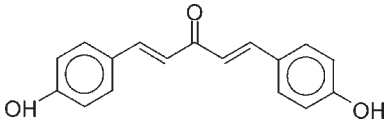
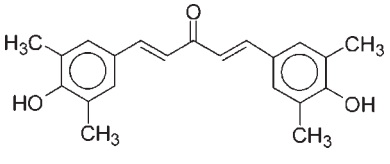
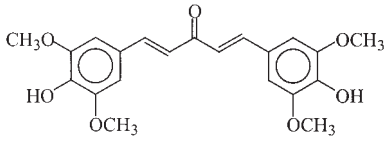
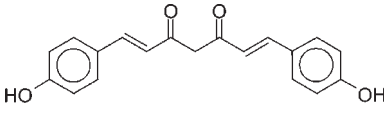
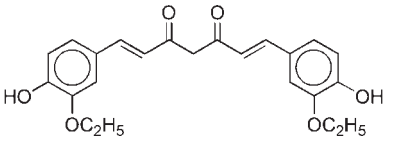
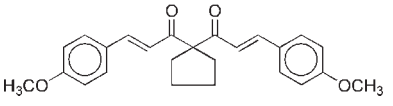
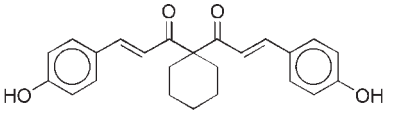
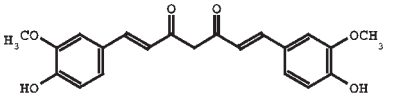
Compound	Structure	Kmin^{-1} × 10^{-2}	r	T 1/2
II$_{12}$		9.32 ± 0.114	– 0.991	16.8
II$_{13}$		6.91 ± 0.325	– 0.998	18.3
II$_{15}$		14.80 ± 113	– 0.991	11.3
III$_1$		5.91 ± 0.130	– 0.992	18.3
III$_5$		11.92 ± 111	–0.995	13.3
IV$_1$		1.41 ± 0.013	– 0.985	19.7
IV$_5$		3.41 ± 0.113	– 0.993	20.3
IV$_6$		1.82 ± 0.124	– 0.996	23.6
V$_1$		2.83 ± 0.328	– 0.994	18.2

Table 1. (continued)

Compound	Structure	Kmin^{-1} × 10^{-2}	r	T 1/2
V_4		1.81 ± 0.052	– 0.996	16.6
VI_1		1.90 ± 0.111	– 0.991	19.3
VII_1		1.33 ± 0.230	– 0.998	21.6
Curcumin		3.30 ± 0.11	–0.998	19.5

K_{app} min^{-1} × 10^{-2} 5.91 and 11.92, respectively. Upon substitution of the cyclic structure either piperidone or cycloheptanone with acetone leads to a remarkable decrease in the free radical scavenging activity; compounds **IV_1**, **IV_5** < **II_{10}**, **II_{11}**, **II_{15}**, and **III_1** with K_{app} min^{-1} × 10^{-2} 1.41, 3.41 < 16.32, 12.41, 14.80, and 5.91, respectively. Upon increasing the length of the unsaturated chain using acetyl acetone instead of acetone, leads to an increase in the activity as shown by compound **V_1** with K_{app} min^{-1} × 10^{-2} 2.83. Upon ommitting the active methylene group in compounds **V_{1-6}** using either cyclopentanone or cyclohexanone, yielding compounds **VI_{1-6}** and **VII_{1-6}**, leads to a decrease in the free radical scavenging activity (Table 1).

ATP chemiluminescence assay

ATP luminescence is a sensitive and precise method for measuring cell viability [28, 29]. Since 1984, a number of groups have used ATP luminescence to test the chemosensitivity of both cell lines and tumors. The TCA-100 method described in this report uses ATP luminescence to measure chemotherapeutic drug sensitivity and resistance of dissociated tumor cells cultured for 6 d in serum-free medium and polypropylene microplates to inhibit the survival of non-neoplastic cells. This method was developed from a previously published technique using medium containing serum and agarose-coated microplates. Both solid tumors and hematological malignancies have been tested with the TCA-100. The high sensitivity allows to replicate the testing of six single agents or drug combinations at seven concentrations with less than two million cells. The system provides dose-response results and is able to detect heterogenity of drug sensitivity between tumors and drug combination effects consistent with clinical experience, to establish the relationship between energy (ATP) use and oxygen metabolite production in human peripheral blood phagocytes. The system is also able to determine the ability of alterations in these parameters to distinguish toxic from metabolic effects on phagocyte function [28, 29]. In this work, normal human phagocytes obtained from healthy male and female volunteers were used and separated by the use of neutrophil isolation medium (NIM) (Cardinal Associates, Santa Fe, NM, USA).

All of the synthesized compounds were tested for their ability to scavenge oxygen free radical produced by peripheral multinuclear neutrophil cells (PMNs) collected from apparently healthy blood donors. Phorpol-12-myristate-13-acetate (PMA), in a final concentration of 2 ng/mL was added to PMNs (5 × 10^5 cells/mL) to stimulate respiratory burst which will be magnified by luminol (10^{-4} M) for measuring in the LKB luminometer. 100 µg

of the prepared drugs were added to detect its effect on the amount of oxygen radical liberated and the percentage of inhibition was calculated.

In this study, the free radical scavenger determination indicates that compounds **II$_4$**, **II$_9$**, **II$_{10}$**, **II$_{11}$**, **V$_1$**, and **V$_4$** show the highest free radical scavenger activity with % inhibition values of 90.71, 91.24, 96.91, 94.26, 99.23, and 99.85 %, respectively compared to that of **curcumin** with a % inhibition value of 99.55. Moreover, compounds **II$_1$**, **II$_3$**, **II$_6$**, **II$_1$**, **II$_{12}$**, **II$_{13}$** and **III$_5$** are also proved to be moderately effective with % inhibition range of 88.19–70.76 %. In addition, a % inhibition range of 65.44–60.23 % are also obtained by compounds **II$_5$**, **III$_1$**, and **V$_2$** (Table 2, Figure 2).

Table 2. Effect of the tested compounds on the chemiluminescence response and cell viability of isolated human peripheral multinuclear neutrophils (PMNs).

Drugs	CL Response (mV)	% Inhibition	% Viability
II$_1$	110.88 ± 18.01	70.76 ± 3.09	64
II$_3$	79.61 ± 15.09	79.01 ± 3.88	70
II$_4$	35.24 ± 5.33	90.71 ± 1.22	81
II$_5$	150.83 ± 3.23	60.23 ± 4.48	88
II$_6$	57.18 ± 6.36	84.92 ± 2.64	85
II$_7$	102.36 ± 13.16	73.01 ± 3.87	86
II$_9$	33.22 ± 4.66	91.24 ± 2.35	68
II$_{10}$	117.72 ± 1.86	96.91 ± 0.88	82
II$_{11}$	21.76 ± 3.60	94.26 ± 1.25	88
II$_{12}$	44.80 ± 5.99	88.19 ± 5.99	81
II$_{13}$	82.41 ± 8.82	78.27 ± 2.32	75
III$_1$	138.70 ± 47.81	63.42 ± 3.52	85
III$_5$	86.99 ± 12.88	77.06 ± 12.88	79
III$_6$	220.43 ± 7.17	41.87 ± 7.78	88
IV$_1$	288.92 ± 73.32	23.81 ± 5.82	81
IV$_3$	245.07 ± 32.91	35.37 ± 9.34	81
IV$_6$	221.87 ± 25.66	41.49 ± 13.04	77
V$_1$	0.58 ± 0.01	99.23 ± 0.10	91
V$_2$	131.04 ± 37.61	65.44 ± 1.53	82
V$_4$	2.93 ± 0.91	99.85 ± 0.02	89
VI$_1$	334.88 ± 78.35	11.69 ± 6.67	75
VI$_3$	336.20 ± 75.65	11.34 ± 6.24	84
VII$_1$	292.14 ± 22.94	9.53 ± 4.92	85
VII$_3$	367.50 ± 78.04	3.09 ± 6.14	76
Curcumin	48.80 ± 0.01	99.55 ± 0.01	92

Values are expressed as mean ± SEM.
PMA = 2 ng/mL; PMNs = 5 × 10^6 cells/mL; Luminol = 10^{-4} M;
Curcumin derivatives = 100 µg/mL

Effect of drugs on the viability of PMNs

To examine whether these substances have a direct cytotoxic effect on PMNs viability, the percentage of viable cells was estimated by the trypan blue exclusion test (0.2 %) of ten incubation periods 30 min each at 37 °C. In this study, the toxicity of the tested compounds was determined by adding equal parts of these compounds and PMNs. Then the cells were incubated under normal culture conditions (37 °C, 5 % CO_2 atmosphere) for 30 minutes. After incubation, a drop of trypan blue stain was added to the mixture and then examined under the microscope, counting about 100 PMNs, taking the percentage of the viable cells (those, which did not take up the stain) versus the dead (stained) cells. Among the tested compounds, compound **V$_1$** showed slight toxicity with a % viability of 91 %, while compounds **II$_4$**, **II$_5$**, **II$_6$**, **II$_7$**, **II$_{10}$**, **II$_{11}$**, **II$_{12}$**, **III$_1$**, **III$_6$**, **IV$_1$**, **IV$_3$**, **V$_1$**, **V$_2$**, **V$_4$**, **VI$_3$**, and **VII$_1$** showed moderate toxicity with % viability range from 89–81 %. The rest of the tested compounds showed a % viability that range from 79–64 % (Table 2, Figure 2).

Conclusion

Upon determining the antioxidant activity for the synthesized compounds, it was found that, most of the these compounds showed a promising activity. Among them, compounds **II$_4$**, **II$_9$**, **II$_{10}$**, **II$_{11}$**, **V$_1$**, and **V$_4$** showed high free radical scavenger activity with % inhibition values of 90.71, 91.24, 96.91, 94.26, 99.23, and 99.85 %, respectively.

Depending on the data of the oxygen free radical scavenger activity of the tested compounds, the following structural requirements for high antioxidative activity are obtained:

a) p-Hydroxy phenolic structure is the most essential component in the antioxidative structure.

b) Electron-donating substituents on the benzene ring at the ortho-position of the phenolic hydroxy group are also required.

c) Omitting the active methylene group in 1,7-bis(substituted phenyl)-1,6-heptadien-3,5-dione **V$_{1-6}$** to afford the corresponding 1,1-bis(substituted cinnamoyl)-cyclopentanes **VI$_{1-6}$** and 1,1-bis(substituted cinnamoyl)cyclohexanes **VII$_{1-6}$**, leads to a decrease in the antioxidative activities.

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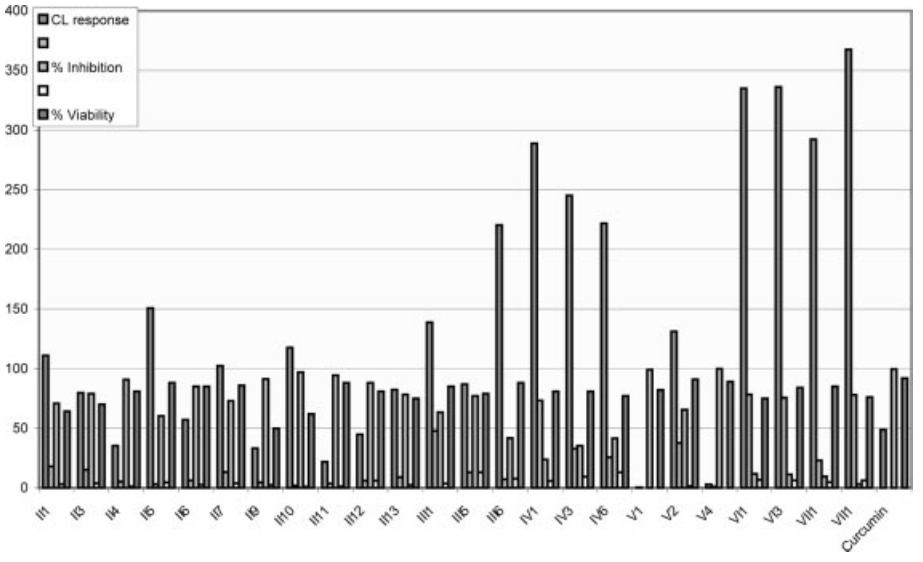

Figure 2. Chemiluminescence response with corresponding % inhibition and % viability of a group of water-soluble curcumin derivatives on isolated human polymorphonuclear leukocytes (PMNs) when stimulated with PMA.

Experimental

Synthesis

Melting points were determined on Mettler FP80 melting point apparatus (England) and are uncorrected. Microanalysis was performed on a Perkin-Elmer 2400 elemental analyzer (Perkin-Elmer, Norwalk, CT, USA) at the Central Research Laboratory, College of Pharmacy, King Saud University. Thin layer chromatography was performed on pre-coated (0.25 mm) silica gel plates, compounds were detected with 254 nm UV lamp, ^{1}H NMR spectra were recorded on a Varian EM 360 (90 MHz) instrument (Varian Inc., Palo Alto, CA, USA) using TMS as internal standard (Chemical shift in δppm). Compounds **II$_2$**, **IV$_2$**, and **IV$_3$** had been synthesized according to a reported procedure [30, 31] (Scheme 2, Table 3).

3,5-bis(substituted benzylidene)-N-alkyl-4-piperidones (II_{1-16}), *2,7-bis(substituted benzylidene)cycloheptanones* (III_{1-5}), *and 1,5-bis(substituted phenyl)-1,4-pentandien-3-ones* (IV_{1-6})

A mixture of the appropriate aldehyde (0.02 mol) and the ketone (0.01 mol) was heated in a water bath at 25–30 °C until a clear solution was obtained, then 2 mL concentrated HCl was added while stirring for 5 min. The reaction mixture was then stirred at room temperature for 2 h. After standing for 2 d, the mixture was treated with cold AcOH/water (1:1) and filtered. The solid obtained was then dried and crystallized from the appropriate solvents (Table 3).

1,7-bis(substituted phenyl)-1,6-heptadien-3,5-diones (V_{1-6})

Acetyl acetone (0.01 mol) and the appropriate aldehyde (0.02 mol) in alcoholic NaOH (50 mL, 10 %) were stirred at room temperature for 10 min. The separated solid was filtered and recrystallized from the suitable solvents (Table 3).

1,7-bis(substituted phenyl)-1,6-heptadien-3,5-diones sodium salts

Compounds **V$_{1-9}$** (0.01 mol) and $NaOCH_3$ (0.04 mol) was stirred under reflux in EtOH for 1 h. The precipitated solid was filtered and used for the following step without further crystallization.

1,1-bis(substituted cinnamoyl)cyclopentane (VI1–6) and 1,1-bis(substituted cinnamoyl)cyclohexane (VII1–6)

The disodium salts of **VI$_{1-6}$** (0.01 mol) were refluxed while stirring with either 1,4-dibromobutane or 1,5-dibromopentane (0.01 mol) in EtOH for 6 h. The precipitated NaBr was filtered, and the filterate was concentrated. The separated compounds were filtered then crystallized from the selected solvent (Table 3).

Biological Testing

Diphenylpicrylhydrazyl free radical test

To a solution of the synthesized compounds (10 mg) in acetonitrile (5 mL), 2,2-diphenyl-1-picrylhydrazyl free radical (3 mL) in acetonitrile (1 mL) was added. The mixture was kept at 37 °C, 0.2 mL were pipetted from the reaction mixture at different time intervals, 5, 10, 15, 25, 30, and 35 min. The decrease of absorption at 515 nm was measured. Form the linear regression line of absorbancy against time, the pseudo-first order rate (K_{app}) and the corresponding half-life time ($t_{1/2}$) of the reaction were calculated (Table 1).

Measurement of oxygen free radicals by chemiluminescence

Isolation of PMNs

Heparinized blood (5–7 mL) was layered over 4 mL of neutrophil isolation medium (NIM) in a round bottom tube and then centrifuged at 400 *g* for 30 min at room temperature. After centrifugation, top layers of plasma and lymphocyte/monocyte were carefully aspirated and the neutrophil layer was removed with a pasteur pipette and transfered to a 15 mL conical centrifuge tube, the tube was filled with HBSS (Hank's balanced salt solution) and mixed to obtain a homogenous suspension which was then centrifuged for l0 min at 350 *g*. After centrifugation, the supernatant was discarded and 2 mL lysation buffer was added and vortexed to resuspend the pellet, the tube was left for 5–10 minutes in ice and then centrifuged for 5 min at 250 *g* and then the supernatant was discarded. The pellets were washed and resuspended with l0 mL HBSS and centrifuged at

Table 3. Physical properties and molecular formula of the synthesized compounds.

Cmpd	X	R	R′	R′′	Solvent	m.p. °C	Yield %	Formulae*	^{1}H NMR (DMSO-d_6)
II$_1$	H	CH_3	H	H	EtOH	197–199	63	$C_{20}H_{19}NO_3$	δ2.20 (s, 3 H, N-CH_3), 2.28–3.32 (m, 4 H,-CH_2NCH_2), 6.73–8.99 (m, 10 H, CH=C and ArH), 9.03 (s, 2 H, OH).
II$_2$	CH_3	CH_3	H	H	EtOH	202–204	65	$C_{22}H_{23}NO_3$	δ 2.21(s, 3 H, N- CH_3), 2.29–3.42 (m, 4 H, -CH_2NCH_2), 3.95 (s, 6 H, OCH_3), 6.98–8.21 (m, 10 H, CH=C and ArH).
II$_3$	H	CH_3	H	OCH_3	EtOH	195–197	69	$C_{22}H_{23}NO_5$	δ 2.20 (s, 3 H, N-CH_3), 2.25–2.35 (m, 4 H, -CH_2NCH_2), 3.45 (s, 6 H, OCH_3), 6.89–7.92 (m, 8 H, CH=C and ArH), 9.53 (s, 2 H, OH).
II$_4$	H	CH_3	H	OC_2H_5	MeOH	231–233	72	$C_{24}H_{27}NO_5$	δ 1.09–1.12 (t, 6 H, CH_2CH_3), 2.23 (s, 3 H, N- CH_3), 2.27–3.34 (m, 4 H, -CH_2NCH_2), 3.69–3.99 (q, 2 H, CH_2CH_3), 7.01–7.99 (m, 8 H, CH=C and ArH).
II$_5$	H	CH_3	CH_3	CH_3	MeOH	183–185	71	$C_{24}H_{24}NO_3$	δ 2.19 (s, 3 H, N-CH_3), 2.28 (s, 12 H, CH_3), 3.03–3.35 (m, 4 H, -CH_2NCH_2), 6.99–8.08 (m, 6 H, CH=C and ArH), 10.5 (s, 2 H, OH).
II$_6$	H	CH_3	OCH_3	OCH_3	Butanol	210–212	67	$C_{28}H_{31}NO_7$	δ 2.23 (s, 3 H, N-CH_3), 3.29–3.45 (m, 4 H, -CH_2NCH_2), 3.95 (s, 6 H, OCH_3), 3.98 (s, 6 H, OCH_3), 7.02–8.19 (m, 8 H, CH=C and ArH), 10.5 (s, 2 H, OH).
II$_7$	H	C_2H_5	H	H	Butanol	108–110	70	$C_{21}H_{25}NO_3$	δ 1.03–1.15 (t, 3 H, CH_2CH_3), 2.30–3.28 (m, 4 H, -CH_2NCH_3), 3.52–3.83 (q, 2 H, CH_2CH_3), 7.03–7.99 (m, 10 H, CH=C and ArH), 9.23 (s, 2 H, OH).
II$_8$	CH_3	C_2H_5	H	H	$CHCl_3$/EtOH	126–128	68	$C_{23}H_{25}NO_3$	δ 1.10–1.14 (t, 3 H, CH_2CH_3), 2.26–3.12 (m, 4 H, -CH_2NCH_3), 3.48–3.82 (q, 2 H, CH_2CH_3), 4.02 (s, 6 H, OCH_3), 7.12–8.01 (m, 10 H, CH=C and ArH).
II$_9$	H	C_2H_5	H	OCH_3	EtOH	>300	75	$C_{23}H_{25}NO_5$	δ 1.03–1.16 (t, 3 H, CH_2CH_3), 2.26–3.38 (m, 4 H, -CH_2NCH_2), 3.96 (s, 6 H, OCH_3), 3.99–4.03 (q, 2 H, CH_2CH_3) 7.04–7.98 (m, 8 H, CH=C and ArH), 9.28 (s, 2 H, OH).
II$_{10}$	H	C_2H_5	H	OC_2H_5	EtOH	238–239	83	$C_{25}H_{29}NO_5$	δ 1.02–1.15 (t, 9 H, CH_2CH_3), 2.28–3.40 (m, 4 H, -CH_2NCH_2), 3.96–4.01 (q, 6 H, CH_2CH_3) 7.02–8.21 (m, 8 H, CH=C and ArH), 9.58 (s, 2 H, OH).
II$_{11}$	H	C_2H_5	CH_3	CH_3	Butanol	180–182	81	$C_{25}H_{29}NO_5$	δ 1.01–1.15 (t, 3 H, CH_2CH_3), 2.23 (s, 12 H, CH_3), 2.28–3.37 (m, 4 H, -CH_2NCH_2), 3.95–4.01 (q, 2 H, CH_2CH_3) 7.03–8.11 (m, 6 H, CH=C and ArH), 9.55 (s, 2 H, OH).

Table 3. (continued)

Cmpd	X	R	R′	R′′	Solvent	m.p. °C	Yield %	Formulae*	^{1}H NMR (DMSO-d_6)
II$_{12}$	H	C_2H_5	OCH_3	OCH_3	Dioxane	>300	73	$C_{29}H_{33}NO_7$	δ 1.02–1.15 (t, 3 H, CH_2CH_3), 2.28–3.34 (m, 4 H, -CH_2NCH_2), 3.95 (s, 6 H, OCH_3), 3.98 (s, 6 H, OCH_3), 7.01–8.11 (m, 4 H, CH=C and ArH), 9.55 (s, 2 H, OH).
II$_{13}$	H	C_3H_7	H	H	Ethanol	198–200	75	$C_{22}H_{23}NO_3$	δ 1.01–1.03 (t, 3 H, CH_3), 1.89–3.11 (m, 6 H, CH_2CH_3 and CH_2NCH_2), 3.63–3.62 (t, 2 H, NCH_2), 7.03–8.52 (m, 15 H, CH=C and ArH), 9.50 (s, 2 H, OH).
II$_{14}$	CH_3	C_3H_7	H	H	$CHCl_3$	191–193	80	$C_{24}H_{27}NO_3$	δ 0.97–1.03 (t, 3 H, CH_3), 1.98–3.21 (m, 6 H, CH_2CH_3 and CH_2NCH_2), 3.23–3.52 (t, 2 H, NCH_2) 3.96 (s, 6 H, OCH_3), 7.03–8.52 (m, 8 H, CH=C and ArH).
II$_{15}$	H	C_3H_7	H	OCH_3	MeOH	168–170	82	$C_{24}H_{27}NO_5$	δ 0.97–1.03 (t, 3 H, CH_3), 1.89–3.11 (m, 6 H, CH_2CH_3 and CH_2NCH_2), 3.23–3.52 (t, 2 H, NCH_2) 3.96 (s, 6 H, OCH_3), 7.03–8.52 (m, 8 H, CH=C and ArH), 9.5 (s, 2 H, OH).
II$_{16}$	H	C_3H_7	H	OC_2H_5	EtOH	232–234	81	$C_{26}H_{31}NO_5$	δ 1.04–1.14 (m, 2 H, $CH_2CH_2CH_3$ 2.09–2.91 (m, 9 H, OCH_2CH_3 and (m, 6 H, CH_2CH_3 and $CH_2CH_2CH_3$), 3.1 (s, 4 H, CH_2NCH_2), 3.41–3.51 (t, 2 H, NCH_2), 3.65–3.69 (q, 4 H, OCH_2CH_3), 7.01–7.98 (m, 8 H, CH=C and ArH), 9.61 (s, 2 H, OH).
III$_1$	H	–	H	H	EtOH	>300	69	$C_{21}H_{20}O_3$	δ 2.12–3.55 (m, 8 H, CH_2), 7.13–7.85 (m, 10 H, CH=C and ArH), 10.5 (s, 2 H, OH), 3.98–4.01 (q, 2 H, CH_2CH_3).
III$_2$	CH_3	–	H	H	$CHCl_3$	175–177	63	$C_{23}H_{24}O_3$	δ 2.02–3.11 (m, 8 H, CH_2), 3.98 (s, 6 H, OCH_3), 7.11–7.92 (m, 10 H, CH=C and ArH), 9.58 (s, 2 H, OH).
III$_3$	H	–	H	OCH_3	EtOH	252–254	65	$C_{23}H_{24}O_5$	δ 2.12–3.41 (m, 8 H, CH_2), 3.96 (s, 6 H, OCH_3), 7.01–7.92 (m, 8 H, CH=C and ArH), 9.88 (s, 2 H, OH).
III$_4$	H	–	H	OC_2H_5	EtOH	166–168	69	$C_{25}H_{28}O_5$	δ 2.11–3.81 (m, 14 H, CH_2 and OCH_2CH_3), 3.50–3.60 (q, 4 H, OCH_2CH_3), 7.22–7.89 (m, 8 H, CH=C and ArH), 9.91 (s, 2 H, OH).
III$_5$	H	–	CH_3	CH_3	$CHCl_3$	89–90	63	$C_{25}H_{28}O_3$	δ 2.14–2.99 (m, 8 H, CH_2), 3.10 (s, 12 H, Ar-CH_3), 6.99–7.88 (m, 6 H, CH=C and ArH), 10.1 (s, 2 H, OH).
IV$_1$	H	–	H	H	MeOH	123–125	65	$C_{17}H_{14}O_3$	δ 7.20–7.93 (m, 12 H, CH=C and ArH), 10.20 (s, 2 H, OH).
IV$_2$	CH_3	–	H	H	Benzene	115–117	74	$C_{19}H_{18}O_3$	δ 3.93 (s, 6 H, OCH_3), 7.11–7.98 (m, 12 H, CH=C and ArH).

Table 3. (continued)

Cmpd	X	R	R′	R′′	Solvent	m.p. °C	Yield %	Formulae*	^{1}H NMR (DMSO-d_6)
IV$_3$	H	–	H	OCH_3	EtOH	129–131	75	$C_{19}H_{18}O_5$	δ 3.39 (s, 6 H, OCH_3), 3.67–3.71 (q, 4 H, *OCH_2*CH_3), 7.03–7.90 (m, 10 H, CH=C and Ar-H), 9.80 (s, 2 H, OH).
IV$_4$	H	–	H	OC_2H_5	EtOH	118–120	69	$C_{21}H_{22}O_5$	δ 2.12–2.33 (t, 6 H, OCH_2*CH_3*), (s, 12 H, CH_3), 6.83–8.17 (m, 6 H, CH=C and ArH), 9.79 (s, 2 H, OH).
IV$_5$	H	–	CH_3	CH_3	EtOH	133–135	75	$C_{21}H_{22}O_3$	δ 2.24 (s, 12 H, CH_3), 6.83–8.17 (m, 6 H, CH=C and ArH), 9.79 (s, 2 H, OH).
IV$_6$	H	–	OCH_3	OCH_3	$CHCl_3$	157–159	70	$C_{25}H_{26}O_7$	δ 3.95 (s, 6 H, OCH_3), 3.97 (s, 6 H, OCH_3), 7.04–8.12 (m, 8 H, CH=C and ArH), 10.3 (s, 2 H, OH).
V$_1$	H	–	H	H	Benzene	91–93	77	$C_{19}H_{15}O_4$	δ 4.34 (s, 2 H, CH_2), 6.99–8.87 (m, 12 H, CH=C and ArH), 9.59 (s, 2 H, OH).
V$_2$	CH_3	–	H	H	Benzene	136–138	45	$C_{21}H_{19}O_4$	δ 3.95 (s, 6 H, OCH_3), 4.58 (s, 4 H, CH_2), 7.21–8.90 (m, 8 H, CH=C and ArH).
V$_3$	H	–	H	OCH_3	EtOH	175–177	45	$C_{21}H_{19}O_6$	δ 3.89 (s, 6 H, OCH_3), 4.13 (s, 2 H, CH_2), 5.99–7.83 (m, 10 H, CH=C and Ar-H), 10.30 (s, 2 H, OH).
V$_4$	H	–	H	OC_2H_5	EtOH	158–160	65	$C_{23}H_{24}O_6$	δ 2.09–2.40 (t, 6 H, OCH_2*CH_3*), 3.52–3.66 (q, 4 H, O*CH_2*CH_3), 4.14 (s, 2 H, CH_2), 6.00–7.98 (m, 10 H, CH=C and Ar-H), 10.20 (s, 2 H, OH).
V$_5$	H	–	CH_3	CH_3	Butanol	140–142	63	$C_{23}H_{24}O_4$	δ 2.20 (s, 2 H, CH_3), 4.23 (s, 2 H, CH_2) 6.85–8.23 (m, 12 H, CH=C and Ar-H), 10.50 (s, 2 H, OH).
V$_6$	H	–	OCH_3	OCH_3	EtOH	135–137	65	$C_{23}H_{24}O_6$	δ 3.98 (s, 6 H, OCH_3), 3.99 (s, 6 H, OCH_3), 4.48 (s, 4 H, CH_2), 7.21–8.90 (m, 8 H, CH=C and ArH) 9.39 (s, 2 H, OH).
VI$_1$	H	–	H	H	$CHCl_3$	112–114	73	$C_{23}H_{22}O_4$	δ 1.21–1.53 (m, 8 H, cyclopentane -CH_2), 5.85–8.02 m, 12 H, CH=C and Ar-H), 9.5 (s, 2 H, OH).
VI$_2$	CH_3	–	H	H	EtOH	102–104	75	$C_{25}H_{26}O_4$	δ 1.35–1.53 (m, 8 H, cyclopentane -CH_2), 3.75 (s, 6 H, OCH_3), 6.68–7.98 (m, 12 H, CH=C and Ar-H).

Table 3. (continued)

Cmpd	X	R	R′	R′′	Solvent	m.p. °C	Yield %	Formulae*	^{1}H NMR (DMSO-d_6)
VI$_3$	H	–	H	OCH_3	MeOH	142–144	63	$C_{25}H_{26}O_6$	δ 1.32–1.56 (m, 8 H, cyclopentane -CH_2), 3.69 (s, 6 H, OCH_3), 6.96–7.88 (m, 12 H, CH=C and Ar-H).
VI$_4$	H	–	H	OC_2H_5	EtOH	173–175	60	$C_{27}H_{30}O_6$	δ 1.22–1.51 (m, 8 H, cyclopentane -CH_2), 2.10–2.55 (t, 6 H, OCH_2CH_3), 3.51–3.69 (q, 4 H, OCH_2CH_3), 6.71–8.00 (m, 12 H, CH=C and Ar-H).
VI$_5$	H	–	CH_3	CH_3	$CHCl_3$	122–124	65	$C_{27}H_{30}O_6$	δ 1.32–1.56 (m, 8 H, cyclopentane -CH_2) 2.21 (s, 12 H, CH_3), 6.85–8.02 (m, 8 H, CH=C and Ar-H), 10.5 (s, 2 H, OH).
VI$_6$	H	–	OCH_3	OCH_3	MeOH	>300	67	$C_{27}H_{30}O_8$	δ 1.30–1.65 (m, 8 H, cyclopentane -CH_2), 3.98 (s, 12 H, OCH_3) 6.93–7.99 (m, 8 H, CH=C and Ar-H), 9.59 (s, 2 H, OH).
VII$_1$	H	–	H	H	MeOH	115–117	85	$C_{24}H_{24}O_4$	δ 1.11–1.43 (m, 10 H, cyclohexane-CH_2) 5.9–7.32 (m, 12 H, CH=C and Ar-H), 10.32 (s, 2 H, OH).
VII$_2$	CH_3	–	H	H	$CHCl_3$	150–152	72	$C_{26}H_{28}O_4$	δ 1.13–1.33 (m, 10 H, cyclohexane- CH_2), 3.98 (s, 6 H, OCH_3) 6.98–7.92 (m, 12 H, CH=C and Ar-H).
VII$_3$	H	–	H	OCH_3	EtOH	180–182	75	$C_{26}H_{28}O_6$	δ 1.13–1.41 (m, 10 H, cyclohexane- CH_2), 3.49 (s, 6 H, OCH_3) 6.00–7.40 (m, 10 H, CH=C and Ar-H), 10.12 (s, 2 H, OH).
VII$_4$	H	–	H	OC_2H_5	EtOH	195–197	74	$C_{28}H_{32}O_6$	δ 1.12–1.45 (m, 10 H, cyclohexane- CH_2), 2.12–2.39 (t, 6 H, OCH_2CH_3), 3.49–3.59 (q, 4 H, OCH_2CH_3), 5.91–7.21 (m, 10 H, CH=C and Ar-H), 9.95 (s, 2 H, OH).
VII$_5$	H	–	CH_3	CH_3	MeOH	201–203	75	$C_{28}H_{32}O_4$	δ 1.14–1.54 (m, 10 H, cyclohexane- CH_2), 2.23 (s, 12 H, Ar-CH_3) 5.98–7.45 (m, 8 H, CH=C and Ar-H), 10.21 (s, 2 H, OH).
VII$_6$	H	–	OCH_3	OCH_3	MeOH	198–200	74	$C_{28}H_{32}O_6$	δ 1.11–1.44 (m, 10 H, cyclohexane- CH_2), 3.92 (q, 4 H, OCH_3), 6.84–7.98 (m, 8 H, CH=C and Ar-H), 9.98 (s, 2 H, OH).

* Analyzed for C, H, N; results were within 0.4 % of the theoretical values for the formulae given.

(250 *g* for 5 min twice. After washing, the pellet was resuspended in 1 mL of 5 % FCS (fetal calf serum) and the neutrophils were counted and adjusted to a final concentration of 5×10^3 cells per mL [29, 30].

Separation of PMNs

Fresh blood was collected by venipuncture from apparently healthy subjects or blood donors in sterile containers with heparin (10 IU/mL, Fischer Scientific Co., Springfield, NJ, USA). The whole blood was then carefully layered over 4 mL of NIM (Neutrophil Isolation Medium, Cardinal Associates) in a labeled 15 mL round bottom tube. Then it was centrifuged at 400 *g* for 30 min at room temperature. The neutrophil layer was carefully removed with a Pasteuripette and transferred to a 15 mL conical centrifuge tube, which was then filled with phosphate buffered saline (PBS, Sigma Chemical Co., St Louis MO, USA) and inverted several times to obtain a homogenous suspension. The tube was centrifuged again at 350 *g* for 10 minutes, then the supernatant was discarded carefully so as not to disturb the pellet. Lysation buffer (2 mL) was added and the tube was vortexed to resuspend the pellet and the suspension was left to stand for 2 minutes at room temperature, after which it was centrifuged again at 250 × g for 5 minutes. The supernatant was discarded and the pellet resuspended with 10 mL of PBS, twice with centrifugation in between washing. After the final washing step, the pellet was resuspended with 1 mL PBS and the neutrophils were counted and adjusted to a final concentration of 5×10^6 cells per mL. Viability was determined by trypan blue (0.2 %) exclusion test, only a PMN viability above 90 % was used in the experiment [29, 30].

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