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Short communication

Synthesis of benzylideneacetophenones and their inhibition of lipid peroxidation

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Abstract – A series of benzylideneacetophenone derivatives have been synthesized and found to show potent inhibition of the lipid peroxidation (LPO) in rat liver microsomes. All 19 compounds prepared in this series are LPO inhibitors. The highest activity was found in *para* hydroxy derivatives with two *meta tert*-butyl substituents. © 2000 Éditions scientifiques et médicales Elsevier SAS

benzylideneacetophenones / chalcones / lipid peroxidation / antioxidant

1. Introduction

Currently no drugs have been introduced which exhibit their anti-inflammatory activity solely based on the radical scavenging or antioxidant effects [1]. However, the antioxidant effect may inhibit the oxidative process leading to inflammation because the antioxidant activity as an elaborate defence system of the cell will blockade radical formation in the membrane. As we know, in the case of inflammation the radical production overwhelms the defence system resulting in reactive oxygen species which play a role in the inflammation process.

A wide assortment of phenolic natural products of various structural classes, such as flavonoids, display many biological properties. Interestingly, many of these compounds, particularly those with anti-inflammatory activity, contain one or more phenolic hydroxyl groups. Various derivatives of benzylidenacetophenone show anti-inflammatory [2, 3], antibacterial [4], antiviral [5], and gastric protectant activities [6].

Sogawa et al. [7] found that benzylidenacetophenones containing a 3,4-dihydroxycinnamoyl structure strongly inhibit lipid peroxidation (LPO). One of the modifications at the aromatic ring which was carried out by Lazer et al. [8] resulted in the structure shown in *figure 1* (R_1 = CH_3, R_2 = F).

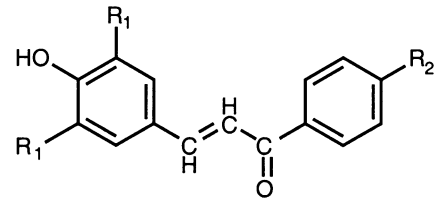

Figure 1. Structure of 1,3-diphenylpropen-1-ones.

This compound has an inhibitory activity on 5-lipoxygenase of 33% at a dose of 1 mM, whereas the inhibitory activity on cyclo-oxygenase was 43% at the same dose. Recently Rajakumar and Rao [9] reported the inhibitory effect on lipid peroxidation using rat brain homogenates of a related compound (*figure 1*, R_1 = *tert* butyl, R_2 = H) with IC_{50} = 9.7 µM.

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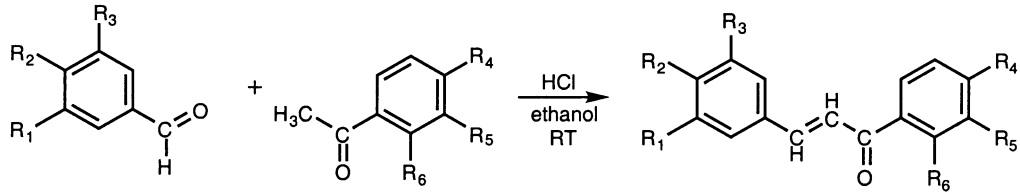


Figure 2. Synthesis of benzylideneacetophenones.

In order to evaluate the inhibition of LPO by this type of compound we prepared a series of benzylidenacetophenones and established their inhibition of LPO.

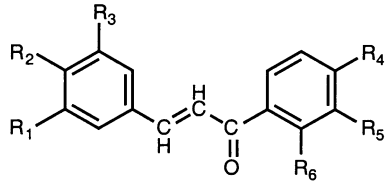
2. Chemistry

Sogawa et al. [7] synthesized 3,4-dihydroxychalcone under basic conditions using the Claisen-Schmidt condensation of acetophenone with 3,4-dihydroxybenzaldehyde. The yield was below 10%. However, when 3,4-dihydroxybenzaldehyde as the bis(tetrahydropyranyl) ether was condensed with acetophenone, the yield was increased to about 45%.

Interestingly, Herdan et al. [10] described the synthesis of a small series of the title compounds under acidic conditions in 75–80% yield.

In our study this improved method was used to synthesize a series of benzylideneacetophenones (*figure 2*). In total, 19 compounds were synthesized from the corresponding benzaldehydes and acetophenones (*table I*). With TLC each isolated product gave a single spot.

Table I. Results of the synthesis of benzylideneacetophenones.



Number	R_1	R_2	R_3	R_4	R_5	R_6	Yield (%)	M.p. (°C)	Crystallized from
1	CH_3O	OH	H	H	H	H	37	85–90	EtOH
2	CH_3O	OH	H	CH_3	H	H	15	115–118	EtOH/H_2O
3	CH_3O	OH	H	CH_3O	H	H	34	161–164	EtOH
4	CH_3O	OH	H	Cl	H	H	13	118–120	EtOH
5	CH_3O	OH	H	H	H	OH	13	128–130	EtOH
6	CH_3O	OH	CH_3O	CH_3	H	H	24	108–110	EtOH
7	CH_3O	OH	CH_3O	CH_3O	H	H	50	148–149	EtOH/H_2O
8	CH_3O	OH	H	OH	CH_3O	H	52	126–128	EtOH/H_2O
9	CH_3O	OH	H	F	H	H	36	94–97	EtOH/H_2O
10	CH_3O	OH	CH_3O	F	H	H	31	130–131	EtOH/H_2O
11	t-Bu	OH	t-Bu	F	H	H	69	125–127[a]	EtOH/H_2O
12	t-Bu	OH	t-Bu	OH	CH_3O	H	59	163–165	EtOH
13	CH_3O	OH	H	OH	H	H	84	124–127	EtOH/H_2O
14	i-Pr	OH	i-Pr	F	H	H	56	147–152	EtOH/H_2O
15	t-Bu	OH	t-Bu	CH_3	H	H	20	147–148[b]	EtOH
16	t-Bu	OH	t-Bu	C_2H_5O	H	H	24	133–135	EtOH
17	t-Bu	OH	t-Bu	CH_3O	H	H	60	152–153[c]	EtOH
18	t-Bu	OH	t-Bu	OH	H	H	32	102–104	EtOH/H_2O
19	t-Bu	OH	t-Bu	Cl	H	H	30	123–124[d]	EtOH

[a] M.p. 117–118° [10]; [b]m.p. 145–147° [10]; [c]m.p. 149–150° [10]; [d]m.p. 123° [10].

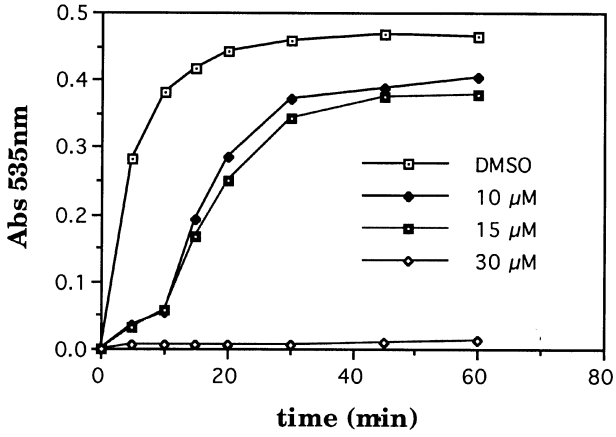


Figure 3. Absorbance at 535 nm versus incubation time in the LPO test using various concentrations of compound **1**.

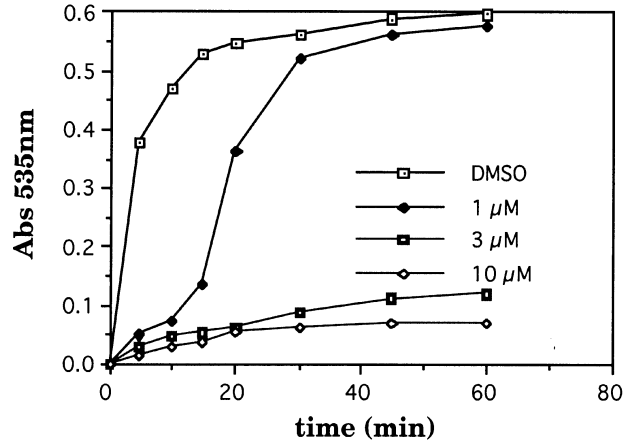


Figure 4. Absorbance at 535 nm versus incubation time in the LPO test using various concentrations of compound **19**.

Structures were confirmed with infrared spectroscopy, nuclear magnetic resonance and mass spectrometry.

3. Pharmacology

Lipid peroxidation is mediated by radicals. The radicals are formed in the inflammatory cascade as part of the defence mechanism of the body. In this process polyunsaturated fatty acids (PUFA's) located in the membrane, are peroxidized via a free radical mechanism. One of the products formed is malondialdehyde (MDA). With thiobarbituric acid it gives a pink colour; its concentration can be measured spectrophotometrically at l = 535 nm and is used as a parameter for lipid peroxidation.

The LPO inhibition test was used to determine the effect of the benzylideneacetophenones prepared on the Fe^{2+}/Vit C induced lipid peroxidation in rat liver microsomes.

In this study, the LPO test was performed non-enzymatically; the interaction between antioxidant with enzymes present in the membranes is prevented by heating the membranes used before incubation. The percentage of inhibition was calculated when the control had reached its maximum level. The profile of the control curve showed that 90% of the maximum absorbance was reached after 15 min of incubation (*figures 3* and *4*).

All the compounds prepared in this series are active as inhibitors of lipid peroxidation; a remarkable common structural feature is the hydroxyl group at the *para* position in the one of the rings.

4. Results and discussion

Concerning the inhibition of LPO, it seems the *para* hydroxyl group of the compounds prepared has the same function as the hydroxyl group of α-tocopherol (*figure 5*). The α-tocopherol molecule can be divided into two parts, a chroman head and a phytyl chain [11].

It is generally accepted that the phytyl chain interchelates with the fatty acid residues of phospholipids, while the chroman head is responsible for the antioxidant effect facing the cytosol, although the chroman ring is still located in the hydrophobic zone of the lipid bilayer [11]. The mechanism of the antioxidant activity of benzylideneacetophenone analogues may involve scavenging of a radical ($R^{\cdot}$) by abstracting a hydrogen atom from the aromatic hydroxyl group at the *para* position (ArOH), thus forming a radical ($ArO^{\cdot}$).

$R^{\cdot} + ArOH \rightarrow RH + ArO^{\cdot}$

The radical ($ArO^{\cdot}$) is fairly stable due to delocalization of the unpaired electron. Compounds with less orbital overlap between a lone pair of the oxygen and the aromatic π-system, were found to be less potent antioxidants compared to tocopherols [12].

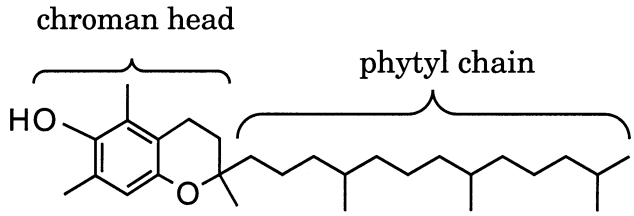
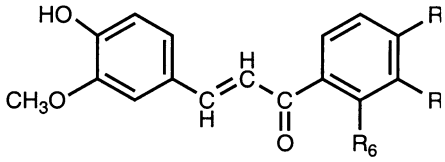


Figure 5. Structure of α-tocopherol.

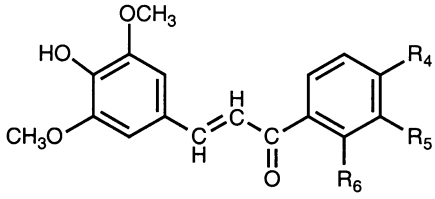
From the profile of the absorbance versus time curve (*figures 3* and *4*), a lag time was observed which is caused by antioxidant consumption.

Based on the differences of the substituents R_1, R_2 and R_3, the synthetic compounds can be classified into four groups as follows:



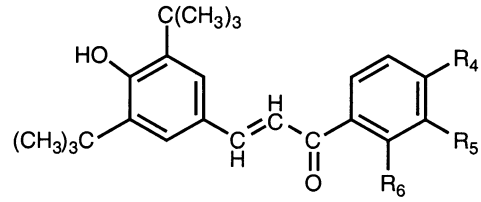
Compounds of group 1

Group 1 contains compounds with substituents R_1 = OCH_3, R_2 = OH and R_3 = H which is the case for compounds **1**, **2**, **3**, **4**, **5**, **8**, **9** and **13**. Their IC_{50} range is 6–20 μM after 15 min incubation time, while after 30 min incubation time their IC_{50} range is 7–22.5 μM. In general, the range of IC_{50} values of compounds in this group after 15 min incubation did not differ from IC_{50} values after 30 min incubation.



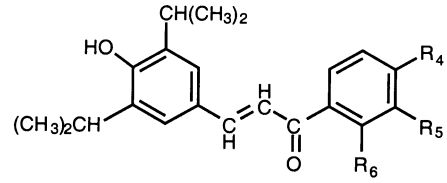
Compounds of group 2

Group 2 contains compounds with substituents R_1 = R_3 = OCH_3 and R_2 = OH which is the case for compounds **6**, **7** and **10**. Their IC_{50} range is 0.2–4.24 μM after 15 min incubation, while after 30 min incubation their IC_{50} range is 1–5 μM. In general, the range of IC_{50} values of compounds in this group after 15 min incubation did not differ from IC_{50} values after 30 min incubation. Compound number **6** (R_4 = CH_3, R_5 = R_6 = H) is the most active compound in this group. After 30 min incubation the IC_{50} value (1.28 μM) of this compound is six times its IC_{50} value (0.21 μM) after 15 min incubation, while for compounds **10** and **7** the ratios are 2 and 1.2, respectively. The substitution of a methyl at R_3 (**6**) by fluor to give compound **10** or methoxy to give compound **7** decreased the inhibitory activity (compounds **6** > **10** > **7**).



Compounds of group 3

Group 3 contains compounds with substituents R_1 = R_3 = t-Bu, R_2 = OH which is the case for compounds **11**, **12**, **15**, **16**, **17**, **18** and **19**. Their IC_{50} range is 0.18–2 μM after 15 min incubation, while after 30 min incubation their IC_{50} range is 1.6–3.6 μM. Variation of R_4 with F and CH_3 did not affect the inhibitory activity after 30 min (**11** ≈ **15**). Variation of R_4 by OCH_3, OC_2H_5, OH or Cl also did not affect the inhibitory activity (compounds **16** ≈ **17** ≈ **18** ≈ **19**). Remarkably, the introduction of an additional OCH_3 group in **18** affording **12** has no influence on the activity. Nevertheless substitutents at R_4 like OCH_3, OC_2H_5, OH or Cl provide, in this small series, the most active compounds (compounds **11** ≈ **15** < **12** ≈ **16** ≈ **17** ≈ **18** ≈ **19**).



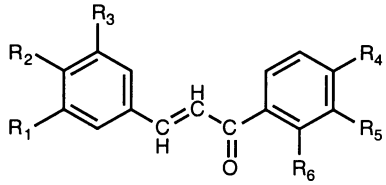
Compounds of group 4

Group 4 may contain compounds with substituents R_1 = R_3 = i-Pr, R_2 = OH, although there is only one example, viz. compound **14**. Its IC_{50} value is 2.43 μM after 15 min incubation, while after 30 min incubation its IC_{50} value is 5.02 μM.

5. Conclusions

LPO inhibitory activities of several compounds in *table II* are in the same order of magnitude as the well-known NDGA. The data mentioned above indicate that the series prepared contained very potent compounds. In more detail, the IC_{50} data showed that introduction of isopropyl or *tert*-butyl groups at positions R_1 and R_3 increases the inhibition of the LPO. This result is in accordance with what has been reported earlier. Particularly the introduction of *tert*-butyl groups *ortho* towards the *para* hydroxyl group has been established as

Table II. Inhibition of lipid peroxidation by benzylideneacetophenones after 15 and 30 min incubation.



Number	R_1	R_2	R_3	R_4	R_5	R_6	LPO $IC_{50}15'$* (μM ± SEM) $n = 2$	LPO $IC_{50}30'$** (μM ± SEM) $n = 2$
1	CH_3O	OH	H	H	H	H	7.10 ± 1.63	17.93 ± 1.50
2	CH_3O	OH	H	CH_3	H	H	6.07 ± 7.25	12.37 ± 0.94
3	CH_3O	OH	H	CH_3O	H	H	17.06 ± 0.30	18.51 ± 1.23
4	CH_3O	OH	H	Cl	H	H	5.06 ± 0.60	7.17 ± 2.50
5	CH_3O	OH	H	H	H	OH	10.55 ± 4.02	14.84 ± 0.62
6	CH_3O	OH	CH_3O	CH_3	H	H	0.21 ± 0.02	1.28 ± 0.51
7	CH_3O	OH	CH_3O	CH_3O	H	H	4.24 ± 0.40	5.06 ± 0.35
8	CH_3O	OH	H	OH	CH_3O	H	18.27 ± 1.06	22.53 ± 2.07
9	CH_3O	OH	H	F	H	H	19.08 ± 0.08	20.44 ± 0.45
10	CH_3O	OH	CH_3O	F	H	H	1.96 ± 0.04	4.17 ± 0.04
11	t-Bu	OH	t-Bu	F	H	H	1.97 ± 0.02	3.61 ± 0.45
12	t-Bu	OH	t-Bu	OH	CH_3O	H	0.55 ± 0.05	1.99 ± 0.02
13	CH_3O	OH	H	OH	H	H	14.28 ± 1.06	19.76 ± 0.11
14	i-Pr	OH	i-Pr	F	H	H	2.43 ± 0.50	5.02 ± 0.56
15	t-Bu	OH	t-Bu	CH_3	H	H	1.84 ± 0.09	3.59 ± 2.24
16	t-Bu	OH	t-Bu	C_2H_5O	H	H	0.19 ± 0.01	1.85 ± 0.03
17	t-Bu	OH	t-Bu	CH_3O	H	H	1.27 ± 0.05	1.95 ± 0.05
18	t-Bu	OH	t-Bu	OH	H	H	0.53 ± 0.05	1.68 ± 0.05
19	t-Bu	OH	t-Bu	Cl	H	H	0.22 ± 0.01	1.82 ± 0.01
20**							0.30 ± 0.01	0.33 ± 0.00

* Incubation time 15 min.
** Incubation time 30 min; reference compound NDGA (nordihydroguaiaretic acid).

favourable for LPO inhibition [13]. However, it is doubtful whether the effect is caused by steric hindrance of the phenolic OH group or by the lipophilic nature of the *tert*-butyl group [13].

Concerning this aspect it is interesting to note that replacement of the two *tert*-butyl groups (group 3) by two methoxy groups (group 2) affords compounds with almost equal LPO inhibiting activity. Since the mono methoxy derivatives (group 1) show much less LPO inhibition, it may be concluded that the presence of two substituents *ortho* to the phenolic OH group is favourable for activity. At least in our series it seems that the electronic nature of the substituents is of less importance.

Variations of R_4, R_5 and R_6 did not show strong effects on LPO inhibitory activity.

6. Experimental protocols

6.1. Chemistry

6.1.1. General remarks

Melting points were obtained using a Mettler FP52 microscope and heating table and are uncorrected. TLC (Merck UC alufolien) experiments were performed with CH_2Cl_2 as mobile phase. IR spectra were obtained from KBr pellets with a Mattson Galaxy 6030 FT-IR apparatus. ^{1}H-NMR spectra were recorded on a Bruker 200 MHz spectrometer with tetramethylsilane as an internal standard. Mass spectra were recorded using a Finnigan MAT 90 with EI ionisation (70 eV).

6.1.2. General procedure

Benzaldehyde derivatives (26.6 mmol) and acetophenone or acetophenone derivatives (30 mmol) were dissolved in 200 mL of ethanol saturated with HCl. HCl and N_2 gas were bubbled into the reaction mixture during the reaction. Stirring was carried out for at least 6 h and each hour the reaction mixture was analysed by TLC. Stirring was stopped when the spot of the reaction product on the TLC plates used was predominantly present. Then the reaction mixture was poured into ice water. The mixture was stirred until some crystals were produced. After standing for 3 h the solid was filtered off and recrystallized using a suitable solvent.

6.1.2.1. 3-(4-Hydroxy-3-methoxyphenyl)-1-phenyl-2-propen-1-one **1**

IR (cm^{-1}): 1 655 (C=O), 3 323 (OH, phenolic), 3 100 (=C–H, alkene; =C–H, aromatic). NMR $CDCl_3$, δ (ppm): 3.95 (s, 3H, -OCH_3), 5.90 (s, 1H, -OH), 7.35 (d, 1H_a, J = 13 Hz, =CH), 7.75 (d, 1H_b, J = 13 Hz, =CH), 7.1 (s, 1H, Ar 2′-H), 6.95 (d, H, Ar 5′-H), 7.2 (d, H, Ar 6′-H), 7.50 (m, 3H, Ar 3″,4″,5″-H), 8.00 (d, 2H, Ar 2″,6″-H). Mass spectrum (EI, *m/z*): 254 ($M^{+\cdot}$, $C_{16}H_{14}O_3^+$, 100%), 253 ($C_{16}H_{13}O_3^+$, 39%), 177 ($C_{10}H_9O_3^+$, 23%), 105 ($C_7H_5O^+$, 34.%), 77 ($C_6H_5^+$, 66%), 51 ($C_4H_3^+$, 27%) mass units. Exact mass of molecular ion: *m/z* = 254.0941, calculated for $C_{16}H_{14}O_3$: 254.0943.

6.1.2.2. 3-(4-Hydroxy-3-methoxyphenyl)-1-(4-methylphenyl)-2-propen-1-one **2**

IR (cm^{-1}): 1 655.0 (-C=O) 3 402.6 (-OH, aromatic), 1 464 and 1 431 (CH_3), 1 583 and 1 514 (-CH=CH-, aromatic). NMR $CDCl_3$, δ (ppm): 2.43 (s, 3H, -CH_3), 3.95 (s, 3H, -OCH_3), 6.00 (s, 1H, -OH), 7.35 (d,1H_a, J = 13 Hz, =CH) 7.75 (d, 1H_b, J = 13 Hz, =CH), 6.95 (d, 2H, Ar 3″,5″-H), 7.92 (d, 2H, Ar 2″,6″-H), 7.28 (s, 1H, Ar 2′-H), 7.15 (m, 2H, Ar 5′,6′-H). Mass spectrum (EI, *m/z*): 269 ($M^{+\cdot}$ + 1, $C_{17}H_{16}O_3^+$, 16.7%), 268 ($M^{+\cdot}$, $C_{17}H_{16}O_3^+$, 100%), 267 ($C_{17}H_{15}O_3^+$, 38%), 177 ($C_{10}H_9O_3^+$, 14.7%), 119 ($C_8H_7O^+$, 32%), 91 ($C_7H_7^+$, 40.9%) mass units. Exact mass of molecular ion: *m/z* = 268.1100, calculated for $C_{17}H_{16}O_3$: 268.1100.

6.1.2.3. 3-(4-Hydroxy-3-methoxyphenyl)-1-(4-methoxyphenyl)-2-propen-1-one **3**

IR (cm^{-1}): 3 418 until 3 352 (-OH, hydroxyl aromatic), 3 100 and 3 025 (-C–H, aromatic), 2 960 and 2 850 (-C–H, aliphatic), 1 651 (-C=O), 1 591 and 1 514 (CH=CH, aromatic). NMR $CDCl_3$, δ (ppm): 3.83 (s, 3H, -OCH_3), 3.93 (s, 3H, -OCH_3), 6.00 (s, 1H, -OH), 7.20 (d, 1H, J = 13 Hz, =CH_a) 7.35 (d, 1H, J = 13 Hz, =CH_b), 6.95 (d, 2H, Ar 3″,5″-H), 8.00 (d, 2H, Ar 2″,6″-H), 7.1 (s, 1H, Ar 2′-H), 6.90 (d, 1H, Ar 5′-H), 7.2 (d,1H, Ar 6′-H). Mass spectrum (EI, *m/z*): 284 ($M^{+\cdot}$, $C_{17}H_{16}O_4^+$, 100%), 283 ($M^{+\cdot}$-H, $C_{17}H_{15}O_4^+$, 28%), 269 ($M^{+\cdot}$-CH_3, $C_{16}H_{13}O_4^+$, 17%), 253 ($M^{+\cdot}$-OCH_3, $C_{16}H_{13}O_3^+$, 16%), 135 ($C_8H_7O_2^+$, 30%) mass units. Exact mass of molecular ion: *m/z* = 284.1048, calculated for $C_{17}H_{16}O_4$: 284.1043.

6.1.2.4. 1-(4-Chlorophenyl)-3-(4-hydroxy-3-methoxyphenyl)-2-propen-1-one **4**

IR (cm^{-1}): 1 651 (C=O), 3 508 (OH, phenolic), 2 950 (=C–H, aliphatic); 3 050 (=C–H, aromatic), 1 579 and 1 512 (-CH=CH-, aromatic). NMR $CDCl_3$, δ (ppm): 3.95 (s. 3H, -OCH_3), 5.95 (s, 1H, -OH), 7.35 (d, 1H, J = 13 Hz, =CH_a), 7.75 (d, 1H, J = 13 Hz, =CH_b) 7.33 (s, 1H, Ar 2′-H), 7.15 (m, 2H, Ar 5′,6′-H), 7.45 (d, 2H, Ar3″,5″-H), 7.95 (d, 2H, Ar 2″,6″-H). Mass spectrum (EI, *m/z*): 290 ($M^{+\cdot}$ + 2, $C_{16}H_{13}O_3Cl^+$, 28.3%), 288 ($M^{+\cdot}$, $C_{16}H_{13}O_3Cl^+$, 100%), 287 ($M^{+\cdot}$-H, $C_{16}H_{12}O_3Cl^+$, 41.5%), 271 (M^+-OH, $C_{16}H_{12}O_2Cl^+$, 15%), 257 ($M^{+\cdot}$-OCH_3, $C_{15}H_{10}O_2Cl^+$, 15%), 253 ($M^{+\cdot}$-Cl, $C_{16}H_{13}O_3^+$, 51%), 139 ($C_7H_4ClO^+$, 26%), 111 ($C_6H_4Cl^+$, 34%) mass units. Exact mass of molecular ion: *m/z* = 288.0548, calculated for $C_{16}H_{13}O_3Cl$: 288.0553.

6.1.2.5. 3-(4-Hydroxy-3-methoxyphenyl)-1-(2-hydroxyphenyl)-2-propen-1-one **5**

IR (cm^{-1}): 1 637 (C=O), 3 414 (OH, phenolic), 1 578 and 1 472 (-CH=CH-, aromatic). NMR $CDCl_3$, δ (ppm): 3.95 (s. 3H, -OCH_3), 5.95 (s, 1H, Ar 4-OH), 7.35 (d, 1H, J = 13 Hz, =CH_a), 7.75 (d, 1H, J = 13 Hz, =CH_b), 7.33 (s, 1H, Ar 2′-H), 7.15 (m, 2H, Ar 4″,5″-H), 7.45 (d, 2H, Ar-3″,6″H), 7.95 (d, 2H, Ar 5′,6′-H), 12.9 (s, 1H, Ar 2″-OH). Mass spectrum (EI, *m/z*): 271 ($M^{+\cdot}$ + 1, $C_{16}H_{14}O_4^+$, 13.59%), 270 ($M^{+\cdot}$, $C_{16}H_{14}O_4^+$, 100%), 269 ($M^{+\cdot}$-H, $C_{16}H_{13}O_4^+$, 51.5%), 253 ($M^{+\cdot}$-OH, $C_{16}H_{13}O^+$, 15%), 239 ($M^{+\cdot}$-OCH_3, $C_{15}H_{11}O_3^+$, 5%) mass units. Exact mass of molecular ion: *m/z* = 270.0890, calculated for $C_{16}H_{14}O_4$: 270.0892.

6.1.2.6. 3-(4-Hydroxy-3,5-dimethoxyphenyl)-1-(4-methylphenyl)-2-propen-1-one **6**

IR (cm^{-1}): 1 655 (C=O), 3 400 (OH, phenolic), 3 050 (=C–H, aromatic), 2 950 (C–H, aliphatic), 1 581 and 1 460 (-CH=CH-, aromatic). NMR $CDCl_3$, δ (ppm): 2.5 (s, 3H, CH_3), 4.00 (s. 6H, -OCH_3), 5.83 (s, 1H, -OH), 7.4 (d, 1H, J = 13 Hz, =CH_a), 7.75 (d, 1H, J = 13 Hz, =CH_b), 6.9 (s, 2H, Ar 2′,6′-H), 7.3 (d, 2H, Ar 3″,5″-H), 7.95 (d, 2H, Ar 2″,6″-H). Mass spectrum (EI, *m/z*): 298 ($M^{+\cdot}$, $C_{18}H_{18}O_4$, 100%), 297 ($M^{+\cdot}$-H, $C_{18}H_{17}O_4^+$, 20%), 267 ($M^{+\cdot}$-OCH_3, $C_{17}H_{15}O_3^+$, 41%), 154 ($C_{10}H_{11}O_3^+$, 13.7%), 119 ($C_8H_7O^+$, 18%), 91 ($C_7H_7^+$, 34%) mass units. Exact mass of molecular ion: *m/z* = 298.1202, calculated for $C_{18}H_{18}O_4$: 298.1205.

6.1.2.7. 3-(4-Hydroxy-3,5-dimethoxyphenyl)-1-(4-methoxy-phenyl)-2-propen-1-one **7**

IR (cm^{-1}): 3 660–3 089 (-OH, hydroxyl aromatic), 3 053 (=C–H, aromatic), 2 928 and 2 964 (-C–H, aliphatic), 1 643 (-C=O), 1 597 and 1 425 (CH=CH, aromatic). NMR $CDCl_3$, δ (ppm): 3.88 (s, 3H, $-OCH_3$), 3.97 (s, 6H, 2 $-OCH_3$), 5.8 (s, 1H, -OH), 7.35 (d,1H, J = 13 Hz, $=CH_a$), 7.7 (d, 1H, J = 13 Hz, $=CH_b$), 6.95 (d, 2H, Ar 3″,5″-H), 8.00 (d, 2H, Ar 2″6″-H), 6.85 (s, 2H, Ar 2′,6′-H). Mass spectrum (EI, *m/z*): 315 ($M^{+\cdot}$ + 1, $C_{18}H_{18}O_5^+$, 16.8%), 314 ($M^{+\cdot}$, $C_{18}H_{18}O_5^+$, 100%), 299 ($M^{+\cdot}$-CH_3, $C_{17}H_{15}O_5^+$, 8.9%), 283 ($M^{+\cdot}$-OCH_3, $C_{17}H_{15}O_4^+$, 35%), 135 ($C_8H_7O_2^+$, 35.2%), 107 ($C_7H_7O^+$, 6.7%), 92 ($C_6H_4O^+$, 16%), 64 ($C_5H_4^+$, 8%) mass units. Exact mass of molecular ion: *m/z* = 314.1158, calculated for $C_{18}H_{18}O_5$: 314.1154.

6.1.2.8. 1,3-Bis(4-hydroxy-3-methoxyphenyl)-2-propen-1-one **8**

IR (cm^{-1}): 1 645 (-C=O) 3 589–3 151 (-OH, aromatic), 3 294 (=C–H, aromatic), 1 464 and 1 431 (CH_3), 1 593 and 1 427 (-CH=CH-, aromatic). NMR $CDCl_3$, δ (ppm): 3.98 (s, 6H, 2-OCH_3), 5.95 (s, 1H, -OH), 6.1 (s, 1H, -OH), 7.35 (d, 1H, J = 13 Hz, $=CH_a$), 7.75 (d,1H, J = 13 Hz, $=CH_b$), 6.98 (s, 1H, Ar 2′-H), 7.1 (d, 1H, Ar 6′-H), 7.6 (s, 1H, Ar 2″-H), 7.62 (d, 2H, Ar 6″-H), 7.15 (d, 1H, Ar 5′-H), 7.22 (d, 1H, Ar 5″-H). Mass spectrum (EI, *m/z*): 301 ($M^{+\cdot}$ + 1, $C_{17}H_{16}O_5^+$, 15.5%), 300 ($M^{+\cdot}$, $C_{17}H_{16}O_5^+$, 100%), 299 ($M^{+\cdot}$-H, $C_{17}H_{15}O_3^+$, 18%), 285 ($M^{+\cdot}$-CH_3, $C_{16}H_{13}O_5^+$, 14.7%), 283 (M^+- OH, $C_{17}H_{15}O_4^+$, 6.8%), 269 ($M^{+\cdot}$-OCH_3, $C_{16}H_{13}O_4^+$, 20.5%), 177 ($C_{10}H_9O_3^+$, 32%), 151 ($C_8H_7O_3^+$, 23.6%). Exact mass of molecular ion: *m/z* = 300.1001, calculated for $C_{17}H_{16}O_5$: 300.0998.

6.1.2.9. 1-(4-Fluorophenyl)-3-(4-hydroxy-3-methoxyphenyl)-2-propen-1-one **9**

IR (cm^{-1}): 1 637 (C=O), 3 439 (OH, phenolic), 1 597 and 1 444 (-C=C-, aromatic). NMR $CDCl_3$, δ (ppm): 3.98 (s. 3H, $-OCH_3$), 5.95 (s, 1H, -OH), 7.25 (d, 1H, J = 13 Hz, $=CH_a$), 7.75 (d, 1H, J = 13 Hz, $=CH_b$), 7.35 (s, 1H, Ar 2′-H), 7.18 (d, 1H, Ar 6′-H), 6.95 (d, 1H, Ar 5′-H), 7.15 (m, 2H, Ar 3″,5″-H), 8.00 (m, 2H, Ar 2″,6″-H). Mass spectrum (EI, *m/z*): 273 ($M^{+\cdot}$ + 1, $C_{16}H_{13}O_3F^+$, 15%), 272 ($M^{+\cdot}$, $C_{16}H_{13}O_3F^+$, 100%), 271 ($M^{+\cdot}$-H, $C_{16}H_{12}O_3F^+$, 45.4%), 255 ($M^{+\cdot}$-OH, $C_{16}H_{12}O_2F^+$, 13.6%), 241 ($C_{15}H_{10}O_2F^+$, 15.7%), 257 ($M^{+\cdot}$-CH_3, $C_{15}H_9O_3F^+$, 6%), 123 ($C_7H_4FO^+$, 44%), 95 ($C_6H_4F^+$, 54.7%) mass units. Exact mass of molecular ion: *m/z* = 272.0846, calculated for $C_{16}H_{13}O_3F$: 272.0849.

6.1.2.10. 1-(4-Fluorophenyl)-3-(4-hydroxy-3,5-dimethoxyphenyl)-2-propen-1-one **10**

IR (cm^{-1}): 1 651 (C=O), 3 439 (OH, phenolic), 3 053(=C–H, aromatic), 2 946 (C–H, alkyl), 1 599 and 1 577 (-C=C-, aromatic). NMR $CDCl_3$, δ (ppm): 3.95 (s. 6H, $-OCH_3$), 5.85 (s, 1H, -OH), 7.22 (d, 1H, J = 13 Hz, $=CH_a$), 7.30 (d, 1H, J = 13 Hz, $=CH_b$), 6.88 (s, 2H, Ar 2′,6′-H), 7.15 (d, 2H, Ar 3″,5″-H), 8.05 (d, 2H, Ar 2″,6″-H). Mass spectrum (EI, *m/z*): 303 ($M^{+\cdot}$ + 1, $C_{17}H_{15}O_4F^+$, 15.1%), 302 ($M^{+\cdot}$, $C_{17}H_{15}O_4F^+$, 100%), 271 ($M^{+\cdot}$-OCH_3, $C_{16}H_{12}O_3F^+$, 50.5%), 123 ($C_7H_4OF^+$, 37.1%), 95 ($C_6H_4F^+$, 45.5%) mass units. Exact mass of molecular ion: *m/z* = 302.095, calculated for $C_{17}H_{15}O_4F$: 302.094.

6.1.2.11. 3-(3,5-Ditertbutyl-4-hydroxyphenyl)-1-(4-fluorophenyl)-2-propen-1-one **11**

IR (cm^{-1}): 1 655 (C=O), 3 537–3 214 (OH, phenolic), 1 601 and 1 568 (-C=C-, aromatic). NMR $CDCl_3$, δ (ppm): 1.50 (s. 18H, *tert*-C_4H_9), 5.65 (s, 1H, -OH), 7.30 (d, 1H, J = 13 Hz, $=CH_a$), 7.75 (d, 1H, J = 13 Hz, $=CH_b$) 7.47 (s, 2H, Ar 2′,6′-H), 7.25 (d, 2H, Ar 3″,5″-H), 8.05 (d, 2H, Ar 2″,6″-H). Mass spectrum (EI, *m/z*): 355 ($M^{+\cdot}$ + 1, $C_{23}H_{27}O_2F^+$, 18.2%), 354 ($M^{+\cdot}$, $C_{23}H_{27}O_2F^+$, 79.3%), 339 ($M^{+\cdot}$-CH_3, $C_{22}H_{24}O_2F^+$, 100%), 297 ($M^{+\cdot}$-C_4H_9, $C_{19}H_{18}O_2F^+$, 9%), 123 ($C_7H_4OF^+$, 93.3%), 95 ($C_6H_4F^+$, 45.6%), 57 ($C_4H_9^+$, 27.5%) mass units. Exact mass of molecular ion: *m/z* = 354.1988 calculated for $C_{23}H_{27}O_2F$: 354.1995.

6.1.2.12. 3-(3,5-Ditertbutyl-4-hydroxyphenyl)-1-(4-hydroxy-3-methoxy-phenyl)-2-propen-1-one **12**

IR (cm^{-1}): 1 655 (C=O), 3 603–3 329 (OH, phenolic); 2 951 (C–H, aliphatic), 1 587 and 1 572 (-C=C-, aromatic). NMR $CDCl_3$, δ (ppm): 1.45 (s. 18H, *tert*-C_4H_9), 3.95 (s, 3H, OCH_3), 5.55 (s, 1H, -OH), 6,13 (s, 1H, -OH), 7.35 (d, 1H, J = 13 Hz, $=CH_a$), 7.80 (d, 1H, J = 13 Hz, $=CH_b$), 7.45 (s, 2H, Ar 2′,6′-H), 7.60 (m, 2H, Ar 2″,6″-H), 7.00 (d, 1H, Ar 5″-H). Mass spectrum (EI, *m/z*): 382 (M^+, $C_{24}H_{30}O_4^+$, 100%), 367 (M^+-CH_3, $C_{23}H_{27}O_4^+$, 56.6%), 325 (M^+- C_4H_9, $C_{20}H_{21}O_4^+$, 12%), 151 ($C_8H_7O_3^+$, 41.4%), 123 ($C_7H_7O_2$, 6.5%) mass units. Exact mass of molecular ion: *m/z* = 382.2147, calculated for $C_{21}H_{30}O_4$: 382.2144.

6.1.2.13. 3-(4-Hydroxy-3-methoxyphenyl)-1-(4-hydroxyphenyl)-2-propen-1-one **13**

IR (cm^{-1}): 1 641 (C=O), 3 600 and 3 321 (OH, phenolic), 1 591 and 1 512 (-C=C-, aromatic). NMR DMSO, δ (ppm): 3.89 (s. 3H, $-OCH_3$), 10.00 (s, 2H, 2 x-OH), 7.6 (d, 1H, J = 13 Hz, $=CH_a$), 7.75 (d, 1H, J = 13 Hz, $=CH_b$), 6.80 (d, 1H, Ar 6′-H), 7.23 (d, 1H, Ar 5′-H), 7.50 (s, 1H, Ar 2′-H), 6.90 (d, 2H, Ar 3″,5″-H, 8.05 (d, 2H, Ar

2″,6″-H). Mass spectrum (EI, *m/z*): 271 (M^+ + 1, $C_{16}H_{14}O_4^+$, 14.56%), 270 (M^+, $C_{16}H_{14}O_4^+$, 100%), 269 (M^+-H, $C_{16}H_{13}O_4^+$, 34.5%), 253 (M^+-OH, $C_{16}H_{13}O^+$, 10.6%), 239 (M^+-OCH_3, $C_{15}H_{11}O_3^+$, 9.8%), 121 ($C_7H_5O_2^+$, 45.9%), 93 ($C_6H_5O^+$, 15.1%) mass units. Exact mass of molecular ion: *m/z* = 270.0895, calculated for $C_{16}H_{14}O_4$: 270.0892.

6.1.2.14. 1-(4-Fluorophenyl)-3-(4-hydroxy-3,5-diisopropyl phenyl)-2-propen-1-one **14**

IR (cm^{-1}): 1 653 (C=O), 3 522–3 246 (OH, phenolic); 1 597 and 1 560 (-C=C-, aromatic). NMR $CDCl_3$, δ (ppm): 1.3 (d, 12H, 4 x-CH_3), 3.15 (septet, 2H, 2 x-CH), 5.28 (s, 1H, -OH), 7.35 (d, 1H, *J* = 13 Hz, =CH_a), 7.78 (d, 1H, *J* = 13 Hz, =CH_b), 7.33 (s, 2H, Ar 2′,6′-H), 7.15 (d, 2H, Ar 3″,5″-H), 8.0 (d, 2H, Ar 2″,6″-H). Mass spectrum (EI, *m/z*): 327 ($M^{+\cdot}$ + 1, $C_{21}H_{23}O_2F^+$, 10.61%), 326 ($M^{+\cdot}$, $C_{21}H_{23}O_2F^+$, 50.4%), 283 ($M^{+\cdot}$-C_3H_7, $C_{18}H_{16}O_2F^+$, 100%), 123 ($C_7H_4OF^+$, 61.3%), 95 ($C_6H_4F^+$, 29%) mass units. Exact mass of molecular ion: *m/z* = 326.1681, calculated for $C_{21}H_{23}O_2F$: 326.1682.

6.1.2.15. 3-(3,5-Ditertbutyl-4-hydroxyphenyl)-1-(4-methylphenyl)-2-propen-1-one **15**

IR (cm^{-1}): 1 653 (C=O), 3 350 (broad, OH, phenolic), 2 958 (-CH, aliphatic), 1 585 and 1 566 (-C=C-, aromatic). NMR $CDCl_3$, δ (ppm): 1.50 (s. 18H, *tert*-C_4H_9), 2.45 (s, 3H, -CH_3), 5.55 (s, 1H, -OH), 7.45 (d, 1H, *J* = 13 Hz, =CH_a), 7.80 (d, 1H, *J* = 13 Hz, =CH_b), 7.50 (s, 2H, Ar 2′, 6′-H), 7.30 (d, 2H, Ar 3″,5″-H), 7.95 (d, 2H, Ar 2″,6″-H). Mass spectrum (EI, *m/z*): 351 ($M^{+\cdot}$ + 1, $C_{24}H_{30}O_2^+$, 27.3%), 350 ($M^{+\cdot}$, $C_{24}H_{30}O_2^+$, 100%), 335 ($M^{+\cdot}$-CH_3, $C_{23}H_{27}O_2^+$, 84.4%), 293 ($M^{+\cdot}$-C_4H_9, $C_{20}H_{21}O_2^+$, 15.4%), 119 ($C_8H_7O^+$, 36%), 91 ($C_7H_7^+$, 17.99%) mass units. Exact mass of molecular ion: *m/z* = 350.2244, calculated for $C_{24}H_{30}O_2$: 350.2246.

6.1.2.16. 3-(3,5-Ditertbutyl-4-hydroxyphenyl)-1-(4-ethoxyphenyl)-2 propen-1-one **16**

IR (cm^{-1}): 1 651 (C=O), 3 470 (broad, OH, phenolic), 2 957 and 2 914 (-CH, aliphatic), 1 587 and 1 566 (-C=C-, aromatic). NMR $CDCl_3$, δ (ppm): 1.50 (s. 18H, *tert*-C_4H_9), 1.45 (t, 3H, -CH_3), 4.15 (q, 2H, -CH_2), 5.55 (s, 1H, -OH), 7.45 (d, 1H, *J* = 13 Hz, =CH_a), 7.8 (d, 1H, *J* = 13 Hz, =CH_b), 7.48 (s, 2H, Ar 2′,6′-H), 6.98 (d, 2H, Ar 3″,5″-H), 8.02 (d, 2H, Ar 2″,6″-H). Mass spectrum (EI, *m/z*), 381 ($M^{+\cdot}$ + 1, $C_{25}H_{32}O_3^+$, 26.1%), 380 ($M^{+\cdot}$, $C_{25}H_{32}O_3^+$, 100%), 365 ($M^{+\cdot}$-CH_3, $C_{24}H_{29}O_3^+$, 46.9%), 351 ($M^{+\cdot}$-C_2H_5, $C_{23}H_{27}O_3^+$, 7.11%), 149 ($C_9H_9O_2^+$, 16.5%), 121 ($C_8H_9O^+$, 15.5%) mass units. Exact mass of molecular ion: *m/z* = 380.2354, calculated for $C_{25}H_{32}O_3$: 380.2351.

6.1.2.17. 3-(3,5-Ditertbutyl-4-hydroxyphenyl)-1-(4-methoxyphenyl)-2-propen-1-one **17**

IR (cm^{-1}): 1 656 (C=O), 3 593 (broad, OH, phenolic), 3 028 (CH, aromatic), 2 960 and 2 912 (-CH, aliphatic), 1 601 and 1 570 (-C=C-, aromatic). NMR $CDCl_3$, δ (ppm): 1.50 (s. 18H, *tert*-C_4H_9), 3.90 (s, 3H, -OCH_3), 5.55 (s, 1H, -OH), 7.38 (d, 1H, *J* = 13 Hz, =CH_a), 7.78 (d, 1H, *J* = 13 Hz, =CH_b), 7.48 (s, 2H, Ar 2′,6′-H), 7.00 (d, 2H, Ar 3″,5″-H), 8.05 (d, 2H, Ar 2″,6″-H). Mass spectrum (EI, *m/z*), 367 ($M^{+\cdot}$ + 1, $C_{24}H_{30}O_3^+$, 19.5%), 366 ($M^{+\cdot}$, $C_{24}H_{30}O_3^+$, 100%), 351 ($M^{+\cdot}$-CH_3, $C_{23}H_{27}O_3^+$, 61%), 335 ($M^{+\cdot}$-OCH_3, $C_{23}H_{27}O_2^+$, 3.55%), 309 ($M^{+\cdot}$-C_4H_9, $C_{20}H_{21}O_3^+$, 7.9%), 135 ($C_8H_7O_2^+$, 34.9%), 107 ($C_7H_7O^+$, 2.4%), 77 ($C_6H_5^+$, 0.2%) mass units. Exact mass of molecular ion: *m/z* = 366.2199, calculated for $C_{24}H_{30}O_3$: 366.2195.

6.1.2.18. 3-(3,5′Ditertbutyl-4-hydroxyphenyl)-1-(4-hydroxyphenyl)-2-propen-1-one **18**

IR (cm^{-1}): 1 649 (C=O), 3 491 (broad, OH, phenolic), 3 003 (CH, aromatic), 2 955 and 2 912 (-CH, aliphatic), 1 591 and 1 566 (-C=C-, aromatic). NMR $CDCl_3$, δ (ppm): 1.50 (s. 18H, *tert*-C_4H_9), 5.58 (s, 1H, -OH), 7.25 (s, 1H, -OH), 7.35 (d, 1H, *J* = 13 Hz, =CH_a), 7.80 (d, 1H, *J* = 13 Hz, =CH_b), 7.50 (s, 2H, Ar 2′,6′-H), 6.95 (d, 2H, Ar 3″,5″-H), 8.00 (d, 2H, Ar 2″,6″-H). Mass spectrum (EI, *m/z*): 353 ($M^{+\cdot}$ + 1, $C_{23}H_{28}O_3^+$, 23.5%), 352 ($M^{+\cdot}$, $C_{23}H_{28}O_3^+$, 100%), 337 ($M^{+\cdot}$-CH_3, $C_{22}H_{25}O_3^+$, 77%), 295 ($M^{+\cdot}$-C_4H_9, $C_{19}H_{19}O_3^+$, 9.46%), 121 ($C_7H_5O_2^+$, 38.5%), 93 ($C_6H_5O^+$, 4.14%), 57 ($C_4H_9^+$, 11.57%) mass units. Exact mass of molecular ion: *m/z* = 352.2039, calculated for $C_{23}H_{28}O_3$: 352.2038.

6.1.2.19. 3-(3,5-Ditertbutyl-4-hydroxyphenyl)-1-(4-chlorophenyl)-2-propen-1-one **19**

IR (cm^{-1}): 1 655 (C=O), 3 551 (broad, OH, phenolic), 2 955 (-CH, aliphatic), 1 591 and 1 574 (-C=C-, aromatic). NMR $CDCl_3$, δ (ppm): 1.50 (s. 18H, *tert*-C_4H_9), 5.62 (s, 1H, -OH), 7.30 (d, 1H, *J* = 13 Hz, =CH_a), 7.78 (d, 1H, *J* = 13 Hz, =CH_b), 7.50 (s, 2H, Ar 2′, 6′-H), 7.48 (d, 2H, Ar 3″,5″-H), 7.98 (d, 2H, Ar 2″,6″-H). Mass spectrum (EI, *m/z*): 372 ($M^{+\cdot}$ + 2, $C_{23}H_{27}O_2Cl^+$, 29.12%), 371 ($M^{+\cdot}$ + 1, $C_{23}H_{27}O_2Cl^+$, 21.93%), 370 ($M^{+\cdot}$, $C_{23}H_{27}O_2Cl^+$, 85.37%), 355 ($M^{+\cdot}$-CH_3, $C_{22}H_{24}O_2Cl^+$, 94.87%), 313 ($M^{+\cdot}$-C_4H_9, $C_{19}H_{18}O_2Cl^+$, 10.7%), 139 ($C_7H_4ClO^+$, 43.21%), 111 ($C_6H_4Cl^+$, 13.9%), 57 ($C_4H_9^+$, 27.9%) mass units. Exact mass of molecular ion: *m/z* = 370.1699, calculated for $C_{23}H_{27}O_2Cl$. 370.1700.

6.2. Pharmacology

6.2.1. Determination of the inhibition of lipid peroxidation

Microsomes obtained from male Wistar rats, 250–350 g, (1 mL cup comprises of 17 mg protein/mL in phosphate buffer) were thawed and assimilated in approximately 30 volumes containing Tris-HCl buffer (50 mM, pH 7.4) and 150 mM KCl. The phosphate buffer was removed by centrifugation at 115 000 *g* for 45 min and then the pellet was heated in a water bath at 100 °C for 2 s. In order to dilute the microsomes to 0.9 mg protein/mL, the pellet was diluted in 18 mL of Tris-HCl buffer (50 mM, pH 7.4, and 150 mM KCl) at 4 °C. The ascorbic acid solution and ferrous sulfate solution had been prepared just before the incubation was started. Test compounds were dissolved in DMSO. The final concentrations of test compound used were 1, 3 and 10 µM in 3 000 µL of the incubation mixture or 10, 15 and 30 µM in 3 000 µL of the incubation mixture. After pre-incubation of the mixture, consisting of 1 805 µL Tris buffer, 1 000 µL microsome, 75 µL test compound and 100 µL (100 µM) ascorbic acid, in plastic incubation tubes for approximately 10 min at 37 °C, the reaction was started by adding Fe^{2+} giving 10 µM final concentration (t = 0). Samples of 0.3 mL were taken out from the incubation mixture at t = 0, 5, 10, 15, 30, 45, and 60 min and the reactions were stopped directly by adding 0.3 mL of the incubation mixture into 2 mL of ice cold mixtures of TBA in TCA (4.16 mg/mL) – BHT in EtOH (1.5 mg/mL) (ratio 10:1) in glass incubation tubes. Then the reaction mixtures were first heated for 15 min at 80 °C and then chilled on ice. The denatured proteins were separated from the mixture by centrifugation at 3 000 rpm for 15 min (4 °C). The absorbance of the supernatant was measured at 535 nm, and the presence of protein was checked at 600 nm [14]. IC_{50} values were calculated by interpolation between two concentrations, one giving less and the other more than 50% inhibition. Experiments were done in duplicate.

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