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10-Hydrocinnamoyl- and 10-cinnamoyl-1,8-dihydroxy-9(10*H*)-anthracenones as inhibitors of leukotriene B_4 biosynthesis and HaCaT cell growth

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Abstract – A series of 10-hydrocinnamoyl and 10-cinnamoyl-1,8-dihydroxy-9(10*H*)-anthracenones has been synthesized and evaluated as inhibitors of leukotriene B_4 (LTB_4) biosynthesis and as antiproliferative agents. In the hydrocinnamoyl series, compounds with phenolic hydroxyl groups were less potent inhibitors of LTB_4 biosynthesis than their non-phenolic analogs, suggesting that a nonspecific redox interaction between the compounds and the active site Fe^{3+} of 5-lipoxygenase or chelation of Fe^{3+} is not responsible for the observed activity. Compounds bearing a 10-cinnamoyl group were somewhat less active than their less rigid dihydrocinnamoyl congeners. Many compounds were also potent inhibitors of the growth of HaCaT cells with IC_{50} values in the submicromolar range. Their activity in this assay was equivalent to that observed with the antipsoriatic anthralin, whereas unspecific cytotoxicity was largely reduced as documented by the activity of lactate dehydrogenase released from cytoplasm of keratinocytes. © Elsevier, Paris

anthracenone / antiproliferative activity / keratinocytes / lactate dehydrogenase release / 5-lipoxygenase

1. Introduction

In our continuing search for antipsoriatic agents, 10-phenylacyl-1,8-dihydroxy-9(10*H*)-anthracenones **1** were identified as antiproliferative agents with inhibitory activity against 5- and 12-lipoxygenase, resulting in reduced biosynthesis of the inflammatory leukotrienes and hydroxyeicosatetraenoic acids [1, 2]. Since anthracenones are well-known generators of oxygen radicals during their auto-oxidation under physiological conditions, these species are thought to be responsible for the inflammatory reactions of the skin associated with the antipsoriatic anthralin (**2**, 1,8-dihydroxy-9(10*H*)-anthracenone) [3, 4]. In order to counteract these proinflammatory mediators, antioxidant structures such as catechol or pyrogallol moieties have been attached to the anthracenone nucleus. A second aspect of anthracenones containing phenolic groups resulted from the 5-LO inhibitory properties of these compounds, as many 5-LO inhibitors act by a redox mechanism [5]. One of the most potent 5-LO inhibitors of the phenylacyl series of anthracenones was the 3,4,5-trihydroxyphenylacetyl analog **1c** with an IC_{50} value of 0.3 μM [1]. By contrast, the 3,4-dihydroxyphenylacetyl analog **1b** was about 40-fold less potent than **1c**, although it contains a catechol moiety. As such, it was expected to act by reducing or chelating the active site Fe^{3+} of 5-LO and thus inactivate the enzyme.

Because of the surprising results obtained with these phenolic analogs, we sought to extend the structure–activity relationships for 10-phenylacyl anthracenones. In a recent study from our laboratories we reported on the biological properties of 10-benzoyl-9(10*H*)-anthracenones, the lowest homologs in this series [6]. In this paper we report the synthesis and biological evaluation of analogs bearing 10-hydrocinnamoyl and 10-cinnamoyl substituents.

2. Chemistry

Molecular modeling studies have revealed that intramolecular hydrogen bonding between the *meta*-

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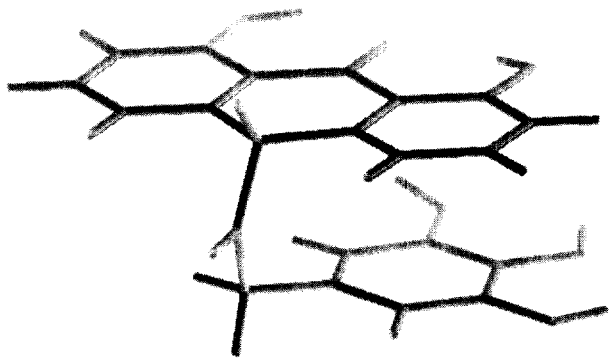


Figure 1. 10-(3,4,5-Trihydroxyphenylacetyl) analog **1c**, molecular mechanics energy minimization in the TRIPOS force field (SYBYL 6.2, Tripos Associates).

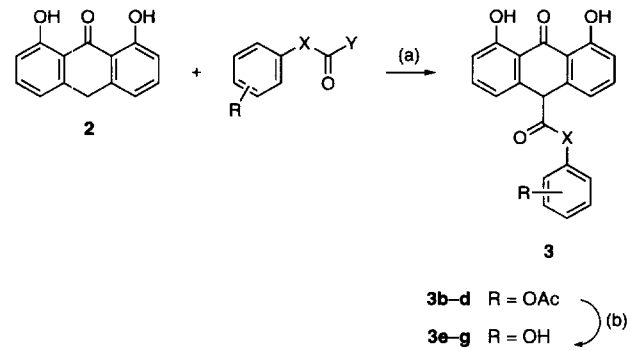


Figure 2. Reagents: (a) Method A, Y = Cl: pyridine, THF, N_2; method B, Y = OH: DCC, pyridine, THF, N_2; (b) K_2CO_3, MeOH, room temperature. R and X are defined in *table I*.

hydroxyl group of **1c** and the 9-carbonyl function of the anthracenone nucleus is enabled (*figure 1*), resulting from a parallel arrangement of the terminal pyrogallol ring of the C-10 substituent and one of the phenyl rings of the anthracenone nucleus through intramolecular van der Waals interaction. Under these conditions, the *meta*-hydroxyl group is masked and would not contribute to the biological action of the compound. At any rate, there are still two neighboring hydroxyl groups available for chelating or reducing the active site Fe^{3+} of the 5-LO enzyme for the pyrogallol analog **1c**. In the case of the catechol **1b**, however, intramolecular hydrogen bonding would result in a structure having only one phenolic hydroxyl group available with respect to Fe^{3+} interaction, comparable to that of the monovalent phenol **1a**. Hence, efficient interaction of **1b** with the target enzyme may be prevented and thus inhibition of leukotriene B_4 (LTB_4) biosynthesis decreased. In support of this, the potency of **1b** against LTB_4 biosynthesis is comparable with that of the 4-hydroxyphenylacetyl analog **1a**.

This prompted us to prepare corresponding analogs with an additional methylene spacer between the terminal phenolic rings and the anthracenone nucleus, in order to disable the above-described intramolecular hydrogen bonding and to further characterize the influence of a catechol/pyrogallol moiety on the biological activity of the compounds. Accordingly, analogs **3b–d** were prepared by reacting **2** with the appropriate acetoxyphenylpropionyl chlorides in THF in the presence of pyridine (*figure 2*). Hydrolysis of these esters using sodium carbonate in methanol at room temperature provided the phenolic analogs **3e–g**. Analogs **3h,i** and the cinnamoyl analogs **3l–p** were directly obtained from the reaction of **2** with the appropriate acids in the presence of the coupling agent dicyclohexylcarbodiimide (DCC).

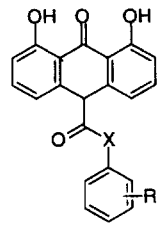
Some of the required analogs were available from previous work, and these are referenced in *table I*. Herein, we also report on their antiproliferative activity and cytotoxicity.

3. Biological results and discussion

The structures of the newly synthesized anthracenones **3** are summarized in *table I*, together with the phenolic phenylacetyl analogs **1a–c** and anthralin **2** as reference compounds. The compounds were evaluated in vitro for inhibition of keratinocyte growth using HaCaT cells [7], a model for highly proliferative epidermis of psoriasis. Proliferation of the keratinocytes was determined directly by counting the dispersed cells under a phase-contrast microscope after 48 h of treatment. In addition to the proliferative aspect of psoriasis, inhibition of LTB_4 biosynthesis was evaluated as a measure of their potency to resolve the inflammatory aspect of the disease. The biosynthesis of LTB_4 was determined in bovine polymorphonuclear leukocytes (PMNL).

In general, substitution at the terminal phenyl ring reduced inhibitory action against LTB_4 biosynthesis as compared to compound **3a** with no substituent. Therefore, a fairly dramatic difference as observed between pyrogallol **1c** and catechol **1b** in the 10-phenylacetyl series could not be seen in the 10-dihydrocinnamoyl series. Among the compounds with a pertinent catechol and a pyrogallol moiety, **3f** and **3g**, only minor differences in inhibition of LTB_4 biosynthesis were obtained. This suggests that a nonspecific redox interaction between the compounds and the active site Fe^{3+} of the 5-LO enzyme or chelation of Fe^{3+} is not the underlying mechanism for the observed inhibition of LTB_4 biosynthesis by these anthracenones. Furthermore, the 10-

Table I. Antiproliferative activity and cytotoxicity against HaCaT cells and inhibition of LTB_4 biosynthesis of 10-hydrocinnamoyl- and 10-cinnamoyl-1,8-dihydroxy-9(10*H*)-anthracenones.



Compound	X	R	AA IC_{50} (μM) [a]	LDH (mU) [b]	LTB_4 IC_{50} (μM) [c]
1a [d]	CH_2	4-OH	1.7	173	14
1b [d]	CH_2	3,4-$(OH)_2$	0.4	194	11
1c [d]	CH_2	3,4,5-$(OH)_3$	1.2	194	0.3
2	anthralin	–	0.7	294	37
3a [d]	$(CH_2)_2$	H	1.6	201	0.5
3b	$(CH_2)_2$	4-OAc	0.8	156	4
3c	$(CH_2)_2$	3,4-$(OAc)_2$	1.4	ND	3
3d	$(CH_2)_2$	3,4,5-$(OAc)_3$	0.8	156	3
3e	$(CH_2)_2$	4-OH	2.7	230	1
3f	$(CH_2)_2$	3,4-$(OH)_2$	0.5	185	4
3g	$(CH_2)_2$	3,4,5-$(OH)_3$	1.6	ND	2
3h	$(CH_2)_2$	3-OMe-4-OH	0.9	185	2
3i	$(CH_2)_2$	3-OH-4-OMe	0.8	171	8
3k [d]	*E*-CH=CH	H	1.0	169	17
3l	*E*-CH=CH	4-OH	1.5	ND	7
3m	*E*-CH=CH	3,4-$(OH)_2$	4.6	ND	6
3n	*E*-CH=CH	3-OMe-4-OH	4.2	ND	9
3o	*E*-CH=CH	3-OH-4-OMe	1.8	ND	13
3p	*E*-CH=CH	3,4-OCH_2O	2.4	ND	16

[a]Antiproliferative activity against HaCaT cells. Inhibition of cell growth was significantly different with respect to that of the control ($N = 3$, $P < 0.01$).
[b]Activity of LDH (mU) release in HaCaT cells after treatment with 2 μM test compound; values are significantly different with respect to vehicle control ($N = 3$, SD < 10%, $P < 0.01$). ND = not determined.
[c]Inhibition of LTB_4 biosynthesis in bovine PMNL. Inhibition was significantly different with respect to that of the control ($N = 3$ or more, $P < 0.01$). Nordihydroguaiaretic acid (NDGA) was used as a standard ($IC_{50} = 0.4$ μM).
[d]See [1].

cinnamoyl derivatives were somewhat less active than their less rigid hydrogenated congeners with a comparable substitution pattern on the phenyl ring, e.g. **3l–o** versus **3e–i**.

Many compounds were potent inhibitors of the growth of HaCaT cells with IC_{50} values less than 1.0 μM. Their activity in this assay was thus equivalent to that observed with the antipsoriatic anthralin. Of interest, the catechol derivatives **1b** and **3f** showed significantly increased antiproliferative activity as compared to their pertinent pyrogallol analogs **1c** and **3g**, respectively. However, the structurally constrained catechol **3m** was a less potent inhibitor of cell growth than the dihydro analog **3f**.

The most potent compounds were also evaluated for release of lactate dehydrogenase into the culture medium [8], as a measure of cytotoxicity. In these experiments, those compounds with antiproliferative activity comparable to that of anthralin showed largely reduced cytotoxicity (**3f,h,i**) or values in the control range (**3b,d**), documenting that their activity was due to cytostatic rather than cytotoxic effects. By contrast, LDH release by anthralin significantly exceeded that of the vehicle control.

4. Experimental protocols

4.1. Chemistry

Melting points were determined with a Büchi 510 melting point apparatus and are uncorrected. Chromatography refers to column

chromatography on silica gel (E. Merck, 70–230 mesh). ^{1}H NMR spectra were recorded with a Varian EM 390 (90 MHz) or Bruker Spectrospin WM 250 spectrometer (250 MHz), using tetramethylsilane as an internal standard. Fourier-transform IR spectra (KBr) were recorded on a Nicolet 510M FTIR spectrometer. Mass spectra (EI) were obtained on a Varian MAT 112S spectrometer (EI-MS, 70 eV). Elemental analyses indicated by the symbols of the elements were within ±0.4% of theoretical values. HPLC (Kontron 420, 735 LC UV detector) was performed on a 250 × 4 mm column (4 × 4 mm precolumn) packed with LiChrospher 100 RP18 (5 µm particles; Merck, Darmstadt, Germany). Data were recorded on a MacLab data acquisition system (WissTech, Germany) and analysis was performed with the software Peaks on an Apple Macintosh computer.

4.1.1. General procedure for the preparation of 10-hydrocinnamoyl- and 10-cinnamoyl-1,8-dihydroxy-9(10H)-anthracenones ***3***

Method A. To a solution of **2** [9] (1.00 g, 4.42 mmol) in absolute THF (30 mL) and dry pyridine (1.00 mL, 12.7 mmol) was added dropwise a solution of the appropriate acyl chloride (5.30 mmol) in absolute THF (10 mL) under N_2.

Method B. To a solution of **2** (1.00 g, 4.42 mmol), the appropriate carboxylic acid (5.30 mmol), and dicyclohexylcarbodiimide (1.10 g, 5.30 mmol) in absolute THF (30 mL) was added dry pyridine (2.0 mL, 25.40 mmol) under N_2.

The reaction mixtures were stirred at room temperature for 4 h, filtered, and the filtrates were evaporated. The residues were purified by chromatography.

4.1.2. 10-[3-(4-Acetoxyphenyl)-1-oxopropyl]-1,8-dihydroxy-9(10H)-anthracenone ***3b***

The title compound was obtained from **2** and 3-(4-acetoxyphenyl)propionyl chloride [10] according to method A. Purification by column chromatography using CH_2Cl_2 gave a yellow powder; 26% yield; m.p. 128–129 °C; FTIR 1753, 1709, 1630 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 12.25 (s, 2H), 7.49–6.72 (m, 10H), 5.16 (s, 1H), 2.64 (t, J = 7.1 Hz, 2H), 2.30 (t, J = 7.1 Hz, 2H), 2.27 (s, 3H); MS m/z = 416 (11, M$^+$). Anal. $C_{25}H_{20}O_6$ (C, H).

4.1.3. 10-[3-(3,4-Diacetoxyphenyl)-1-oxopropyl]-1,8-dihydroxy-9(10H)-anthracenone ***3c***

The title compound was obtained from **2** and 3-(3,4-diacetoxyphenyl)propionyl chloride [11] according to method A. Purification by column chromatography using CH_2Cl_2 and recrystallization from CH_2Cl_2/hexane (7 + 3) gave yellow crystals; 21% yield; m.p. 127–129 °C; FTIR 1773, 1713, 1632 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 12.27 (s, 2H), 7.51–6.63 (m, 9H), 5.15 (s, 1H), 2.70–2.25 (m, 4H), 2.25 (s, 6H). Anal. $C_{27}H_{22}O_8$ (C, H).

4.1.4. 10-[3-(3,4,5-Triacetoxyphenyl)-1-oxopropyl]-1,8-dihydroxy-9(10H)-anthracenone ***3d***

The title compound was obtained from **2** and 3-(3,4,5-triacetoxyphenyl)propionyl chloride (prepared [11] from trihydroxyphenylpropionic acid [12]) according to method A. Purification by column chromatography using CH_2Cl_2/ether (95 + 5) and recrystallization from CH_2Cl_2/hexane (7 + 3) gave a yellow powder; 18% yield; m.p. 142–144 °C; FTIR 1777, 1713, 1632 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 11.93 (s, 2H), 7.61–6.90 (m, 8H), 5.69 (s, 1H), 3.08 (t, J = 7.1 Hz, 2H), 2.66 (t, J = 7.1 Hz, 2H), 2.26 (s, 9H); MS m/z = 532 (8, M$^+$). Anal. $C_{29}H_{24}O_{10}$ (C, H).

4.1.5. General procedure for the preparation of 1,8-dihydroxy-10-[3-(hydroxyphenyl)-1-oxopropyl]-9(10H)-anthracenones

To a solution of the appropriate 10-[3-(acetoxyphenyl)-1-oxopropyl]-1,8-dihydroxy-9(10*H*)-anthracenone (0.60 mmol) in methanol (30 mL) was added K_2CO_3 (3.00 mmol), and the mixture was stirred at room temperature until the hydrolysis was completed (TLC-control). The mixture was neutralized with trifluoroacetic acid, the solvent was removed in vacuo, and the residue was purified by chromatography.

4.1.6. 1,8-Dihydroxy-10-[3-(4-hydroxyphenyl)-1-oxopropyl]-9(10H)-anthracenone ***3e***

The title compound was prepared from **3b**. Purification by column chromatography using CH_2Cl_2/ether (9 + 1) and recrystallization from benzene/ethanol (8 + 2) gave yellow crystals; 80% yield; m.p. 185–189 °C (dec.); FTIR 3347, 1688, 1634 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 11.91 (s, 2H), 9.11 (s br, 1H), 7.61–6.55 (m, 10H), 5.67 (s, 1H), 2.91 (t, J = 7.35 Hz, 2H), 2.49 (t, J = 7.35 Hz, 2H). Anal. $C_{23}H_{18}O_5$ (C, H).

4.1.7. 1,8-Dihydroxy-10-[3-(3,4-dihydroxyphenyl)-1-oxopropyl]-9(10H)-anthracenone ***3f***

The title compound was prepared from **3c**. Purification by column chromatography using CH_2Cl_2/ether (9 + 1) and recrystallization from benzene/ethanol (8 + 2) gave yellow crystals; 59% yield; m.p. 197–202 °C (dec.); FTIR 3407, 1725, 1634 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 11.91 (s, 2H), 9.20–8.20 (br, 2H), 7.61–6.25 (m, 9H), 5.66 (s, 1H), 2.88 (t, J = 7.4 Hz, 2H), 2.44 (t, J = 7.4 Hz, 2H); MS m/z = 390 (5, M$^+$). Anal. $C_{23}H_{18}O_6$ (C, H).

4.1.8. 1,8-Dihydroxy-10-[3-(3,4,5-trihydroxyphenyl)-1-oxopropyl]-9(10H)-anthracenone ***3g***

The title compound was prepared from **3d**. Purification by column chromatography using CH_2Cl_2/ether (8 + 2) and recrystallization from benzene/ethanol (8 + 2) gave a yellow powder; 57% yield; m.p. 221–225 °C (dec.); FTIR 3430, 1719, 1632 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 11.92 (s, 2H), 9.10–8.10 (br, 3H), 7.62–6.96 (m, 6H), 5.97 (s, 2H), 5.67 (s, 1H), 2.85 (t, J = 7.4 Hz, 2H), 2.36 (t, J = 7.4 Hz, 2H); MS m/z = 406 (1.7, M$^+$). Anal. $C_{23}H_{18}O_7$ (C, H).

4.1.9. 1,8-Dihydroxy-10-[3-(4-hydroxy-3-methoxyphenyl)-1-oxopropyl]-9(10H)-anthracenone ***3h***

The title compound was obtained from **2** and 3-(4-hydroxy-3-methoxyphenyl)propionic acid [13] according to method B. Purification by column chromatography using CH_2Cl_2/ether (95 + 5) and recrystallization from benzene gave yellow crystals; 13% yield; m.p. 149–152 °C; FTIR 3408, 1713, 1630 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 11.92 (s, 2H), 9.10–8.30 (s br, 1H), 7.60–6.39 (m, 9H), 5.67 (s, 1H), 3.66 (s, 3H), 2.94 (t, J = 7.3 Hz, 2H), 2.52 (t, J = 7.3 Hz, 2H). Anal. $C_{24}H_{20}O_6$ (C, H).

4.1.10. 1,8-Dihydroxy-10-[3-(3-hydroxy-4-methoxyphenyl)-1-oxopropyl]-9(10H)-anthracenone ***3i***

The title compound was obtained from **2** and 3-(3-hydroxy-4-methoxyphenyl)propionic acid [14] according to method B. Purification by column chromatography using CH_2Cl_2/ether (95 + 5) and recrystallization from benzene gave yellow crystals; 18%

yield; m.p. 143–145 °C; FTIR 3388, 1713, 1634 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.91 (s, 2H), 9.20–8.30 (s br, 1H), 7.61–6.37 (m, 9H), 5.66 (s, 1H), 3.70 (s, 3H), 2.90 (t, J = 7.35 Hz, 2H), 2.47 (t, J = 7.35 Hz, 2H). Anal. $C_{24}H_{20}O_6$ (C, H).

4.1.11. E-1,8-Dihydroxy-10-[3-(4-hydroxyphenyl)-1-oxo-2-propenyl]-9(10H)-anthracenone ***3l***

The title compound was obtained from **2** and 4-hydroxycinnamic acid according to method B. Purification by column chromatography using CH_2Cl_2/ether (95 + 5) and recrystallization from benzene/THF (9 + 1) gave beige crystals; 12% yield; m.p. 218–220 °C (dec.); FTIR 1651, 1634 cm^{-1}; ^1H NMR (DMSO-d_6) δ 12.00 (s, 2H), 10.20 (s, 1H), 7.79 (d, J = 15.8 Hz, 1H), 7.63–6.79 (m, 10H), 6.81 (d, J = 15.8 Hz, 1H), 5.99 (s, 1H); MS m/z = 372 (0.6, M^+). Anal. $C_{23}H_{16}O_5$ (C, H).

4.1.12. E-1,8-Dihydroxy-10-[3-(3,4-dihydroxyphenyl)-1-oxo-2-propenyl]-9(10H)-anthracenone ***3m***

The title compound was obtained from **2** and 3,4-dihydroxycinnamic acid according to method B. Purification by column chromatography using CH_2Cl_2/ether (9 + 1) and recrystallization from benzene/THF (8 + 2) gave a yellow powder; 10% yield; m.p. 225–228 °C (dec.); FTIR 1630 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.99 (s, 2H), 9.78 (s, 1H), 9.20 (s, 1H), 7.69 (d, J = 15.75 Hz, 1H), 7.63–6.75 (m, 9H), 6.69 (d, J = 15.75 Hz, 1H), 5.99 (s, 1H); MS m/z = 388 (0.6, M^+). Anal. $C_{23}H_{16}O_6$ (C, H).

4.1.13. E-1,8-Dihydroxy-10-[3-(4-hydroxy-3-methoxyphenyl)-1-oxo-2-propenyl]-9(10H)-anthracenone ***3n***

The title compound was obtained from **2** and 4-hydroxy-3-methoxycinnamic acid according to method B. Purification by column chromatography using CH_2Cl_2/ether (95 + 5) and recrystallization from benzene gave a yellow powder; 7% yield; m.p. 200–202 °C (dec.); FTIR 3372, 1672, 1630 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.96 (s, 2H), 9.81 (s, 1H), 7.83 (d, J = 15.8 Hz, 1H), 7.63–6.75 (m, 9H), 6.93 (d, J = 15.8 Hz, 1H), 6.08 (s, 1H), 3.83 (s, 3H); MS m/z = 402 (0.7, M^+). Anal. $C_{24}H_{18}O_6$ (C, H).

4.1.14. E-1,8-Dihydroxy-10-[3-(3-hydroxy-4-methoxyphenyl)-1-oxo-2-propenyl]-9(10H)-anthracenone ***3o***

The title compound was obtained from **2** and 3-hydroxy-4-methoxycinnamic acid according to method B. Purification by column chromatography using CH_2Cl_2/ether (95 + 5) gave yellow needles; 21% yield; m.p. 210–212 °C (dec.); FTIR 3295, 1665, 1632 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.99 (s, 2H), 9.70–8.70 (br, 1H), 7.73 (d, J = 15.8 Hz, 1H), 7.64–6.97 (m, 9H), 6.78 (d, J = 15.8 Hz, 1H), 5.76 (s, 1H), 3.82 (s, 3H). Anal. $C_{24}H_{18}O_6$ (C, H).

4.1.15. E-1,8-Dihydroxy-10-[3-(3,4-methylenedioxyphenyl)-1-oxo-2-propenyl]-9(10H)-anthracenone ***3p***

The title compound was obtained from **2** and 3,4-methylenedioxycinnamic acid according to method B. Purification by column chromatography using CH_2Cl_2 and recrystallization from benzene gave yellow needles; 18% yield; m.p. 210–212 °C (dec.); FTIR 1675, 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ 12.33 (s, 2H), 7.58–5.95 (m, 11H), 5.35 (s, 1H), 1.56 (s, 2H). Anal. $C_{24}H_{16}O_6$ (C, H).

4.2. Biological assay methods

The procedures for the biological assays presented in *table I* were described previously in full detail: inhibition of LTB_4 biosynthesis [1], inhibition of HaCaT cell proliferation [15], and release of LDH into culture medium [8].

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