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Original article

10-Phenylbutyryl-substituted anthracenones as inhibitors of keratinocyte growth and LTB_4 biosynthesis

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Abstract – A recent observation that phenylbutyryl anthracenone **2**, an analogue of the antipsoriatic anthralin, is a potent inhibitor of leukotriene B_4 (LTB_4) biosynthesis has prompted a search of other anthracenones with improved antiproliferative activity. In that direction, a limited number of analogues related to **2** have been prepared and evaluated in the HaCaT keratinocytes proliferation and in the polymorphonuclear leukocyte LTB_4 assay. The 4-methoxy analogue **2a** and the side chain methylated **2l** retain the full inhibitory activity of **1** against LTB_4 biosynthesis while their antiproliferative activity is markedly enhanced and comparable to that of the antipsoriatic anthralin. In contrast to anthralin, cytotoxic effects against cell membranes are strongly reduced as documented by the LDH activity released from cytoplasm of keratinocytes. © 2001 Editions scientifiques et médicales Elsevier SAS

anthracenone / antiproliferative activity / HaCaT / hydroxyl radicals / lactate dehydrogenase release / LTB_4 biosynthesis

1. Introduction

Anthracenone derivatives such as anthralin (**1**, *figure 1*) are important agents for the treatment of psoriasis [1]. In contrast to novel agents such as vitamin D and vitamin A derivatives, anthralin not only reduces scales and thickness but is also able to bleach out the erythema [2]. Since its therapeutic use is accompanied by severe inflammation of the non-affected skin, the development of a topically active analogue which should obviate this drawback is highly desirable [3]. As the mechanism of the proinflammatory action of anthracenones is associated with the formation of oxygen radicals by these agents [4], our strategy to overcome this problem was to modulate the oxygen-radical-generating intensity by modifying the critical 10-position of the pharmacophore [5–8]. The phenylbutyryl analogue **2** has been shown to be a potent inhibitor of leukotriene B_4 (LTB_4) biosynthesis with diminished oxygen-radical-producing intensity, and it was clearly demonstrated that the terminal phenyl ring is required for high potency [9].

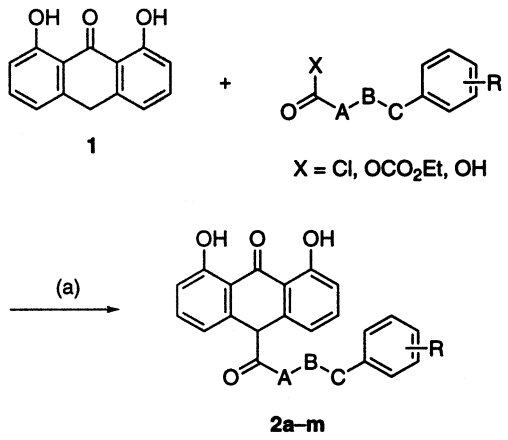

Figure 1. Reagents: (a) Method A, X = Cl: pyridine, toluene, N_2; Method B, X = OCO_2Et: pyridine, acetone, N_2; Method C, X = OH: DCC, pyridine, THF, N_2. A, B, C and R are defined in *table I*.

Abbreviations: LTB_4, leukotriene B_4; LDH, lactate dehydrogenase; MDA, malondialdehyde; PMNL, polymorphonuclear leukocytes.

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This paper describes the continuation of our structure–activity relationship investigations into this new series with particular emphasis on modifications to the chain bearing the terminal phenyl ring. Antipsoriatic anthracenones described to date are achiral and we now wish to report the effect of chiral members of this series on the biological activity.

2. Chemistry

Analogues **2a,b,f–h** were prepared by reacting **1** with the appropriate acyl chlorides in the presence of pyridine (*figure 1*, Method A). This method could not be applied to obtain the 4-oxo-butyryl derivatives **2d,e** of this series since reaction of the 4-oxo-butyric acids with thionyl chloride did not give the required acyl chlorides, but rather cyclised to an unsaturated five-ring lactone. Accordingly, acylation of **1** to give **2d,e** was performed via mixed anhydrides (Method B), which were obtained from the corresponding acids and ethyl chloroformate. As with the required phenylacyl chlorides, which were prepared according to standard literature methods, the crude products were used in the subsequent acylation steps. In an alternative approach (Method C), analogues **2c,i,k,m** were obtained directly from the phenylalkylcarboxylic acids and **1** by the use of the coupling agent dicyclohexylcarbodiimide (DCC) [10]. Finally, the pure enantiomers **2g** and **2h** were prepared from the optically pure (*R*)- and (*S*)-3-phenylbutyric acids, respectively, with an ee $\geq 98\%$ as documented by HPLC analysis on a chiral support.

3. Pharmacology

The novel analogues were evaluated in vitro for inhibition of the growth of HaCaT keratinocytes [11], 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.

Furthermore, a major concern in the testing of potential inhibitors of cell growth is to confirm that the drug does not interfere with the functioning of cell membrane by causing leakage of cytoplasm through it. Accordingly, cytotoxicity against the cell cultures by the potent cell-growth inhibitors was assessed by the activity of lactate dehydrogenase (LDH) released into the culture medium [12].

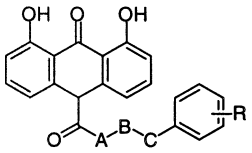
While antiproliferative action in cell cultures may be critical in the management of the proliferative nature of psoriasis, inhibition of the LTB_4 biosynthesis was evaluated as a measure of the potency to resolve the inflammatory aspect of the disease. Although still controversial [13], there is substantial data that supports the involvement of leukotrienes in the amplification of inflammatory reactions in psoriasis [14], and compounds that interfere with their biosynthesis or antagonise their action are explored as potential antipsoriatic agents [15, 16].

Further studies were performed on the redox behaviour of the new compounds using the deoxyribose assay, which is a sensitive test for the production of hydroxyl radicals [17]. The release of thiobarbituric acid reactive material is expressed as malondialdehyde (MDA). It is a measure for hydroxyl-radical generation and thus reflects prooxidant properties of the anthracenones [9].

4. Results and discussion

The structures and biological activity of the novel anthracenones **2a–m** are listed in *table I*, together with the straight chain phenylbutyryl analogue **2** and parent anthralin **1** as reference compounds. From an earlier investigation of the structure–activity relationships of anthracenones for inhibition of 5-lipoxygenase [9], the phenylbutyryl derivative **2** was two orders of magnitudes more potent than the parent **1**. However, as can be seen from the results in *table I*, this study shows that improvement of the LTB_4 inhibitory action is accompanied by a twofold decrease in antiproliferative activity. As a 4-methoxy substituent was beneficial for antiproliferative activity in the phenylacetyl series, this feature was also added to compound **2**. The 4-methoxy analogue **2a** demonstrates that the potent antiproliferative activity of **1** could be regained without sacrificing potent LTB_4 inhibitory properties of **2**. Moreover, an extra methoxy group as in **2b** even has slightly improved antiproliferative activity; however, the potency against LTB_4 biosynthesis was strongly reduced. Introduction of a further keto group into the side chain linking the anthracenone nucleus and the terminal phenyl ring (**2d**) resulted in decrease of both antiproliferative and LTB_4 inhibitory activities. Although the pertinent 4-methoxy analogue **2e** retained the potency against cell growth, inhibitory action against LTB_4 biosynthesis was further decreased.

Table I. Antiproliferative activity and cytotoxicity against HaCaT cells, inhibition of LTB_4 biosynthesis in bovine PMNL, and deoxyribose degradation of 1,8-dihydroxy-10-phenylbutyryl-9(10*H*)-anthracenones and homologues.



Compound	A	B	C	R	AA [a] IC_{50} (μM)	LDH [b] (mU)	LTB_4 [c] IC_{50} (μM)	DD ($\dot{O}H$) [d]
1	Anthralin				0.7	294	37	2.89 ± 0.14 [e]
2	CH_2	CH_2	CH_2	H	1.5	167	0.3	<0.3
2a	CH_2	CH_2	CH_2	4-OMe	0.9	188	0.3	0.56 ± 0.11 [e]
2b	CH_2	CH_2	CH_2	3,4-$(OMe)_2$	0.5	183	15	0.59 ± 0.07 [e]
2c	CH_2	CH_2	CH_2	4-Me	1.0	174	1	ND
2d	CH_2	CH_2	CO	H	1.6	172	8	<0.3
2e	CH_2	CH_2	CO	4-OMe	0.9	130 [f]	20	<0.3
2f	CH_2		$CHCH_3$ (*R*,*S*)	H	0.8	146 [f]	1.5	<0.3
2g	CH_2		$CHCH_3$ (*R*)	H	0.8	ND	2	<0.3
2h	CH_2		$CHCH_3$ (*S*)	H	0.4	ND	1.5	<0.3
2i	CH_2		$CHCH_3$ (*R*,*S*)	4-OMe	1.7	196	0.8	ND
2k	CH_2		$CHCH_3$ (*R*,*S*)	4-Me	1.3	149 [f]	2	ND
2l	CH_2	CH_2	$CHCH_3$ (*R*,*S*)	H	0.8	171	0.3	<0.3
2m	CH_2		$CHCH_2CH_3$ (*R*,*S*)	H	0.8	253	2	ND

[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 ($N = 3$, SD < 10%; controls 149 ± 13).
[c] Inhibition of LTB_4 biosynthesis in bovine polymorphonuclear leukocytes. Inhibition was significantly different with respect to that of the control; $N = 3$ or more, $P < 0.01$. Nordihydroguaiaretic acid (NDGA) was used as the standard inhibitor ($IC_{50} = 0.4$ μM).
[d] Deoxyribose degradation as a measure of hydroxyl-radical formation. Indicated values are μmol of malondialdehyde/mmol of deoxyribose released by 75 μM test compound (control < 0.1).
[e] Values are significantly different with respect to vehicle control.
[f] Values are not significantly different with respect to vehicle control. ND = not determined.

Compounds **2f**–**m** illustrate the influence of branched side chains. The racemic **2f**, an isomer of **2**, exhibited potency against cell growth comparable with **1**, while inhibition of LTB_4 biosynthesis was somewhat weaker than that of **2**. The higher homologues **2l,m** of **2f**, which were also tested as racemic mixtures, showed equally potent antiproliferative activity, and in the case of **2l** even the full inhibitory action of **2** against LTB_4 biosynthesis was retained.

The pure enantiomers **2g,h** of the racemic **2f** were synthesised to explore the effect of chirality on the biological activity of the anthracenone class of antipsoriatic agents. With respect to LTB_4 inhibition, no appreciable difference in potency was observed as compared with the racemic **2f**. This is in contrast with other chiral inhibitors of LTB_4 biosynthesis for which enantioselectivity has been reported [18, 19]. However, in the HaCaT proliferation assay, the (*S*)-enantiomer was twofold more potent than the (*R*)-enantiomer.

As a final study, we examined the cytotoxic effects of the novel anthracenones against the cell membrane. Parent **1** has been reported to cause membrane damage, but did not directly lead to substantial membrane destruction [12]. In this assay, LDH release by **1** as a measure of its potential to induce membrane damage significantly exceeded that of the vehicle control, whereas the activity of the novel compounds was due to cytostatic rather than cytotoxic effects, as LDH release was unchanged or only slightly enhanced as compared to controls. Similar results were obtained in the deoxyribose degradation assay, as a measure of hydroxyl-radical generation. While **1** is a potent hydroxyl-radical generator, deoxyribose degradation by

the novel analogues was either decreased to a minimum or only slightly enhanced as compared to controls, as observed for **2a,b**.

5. Conclusions

In conclusion, 4-methoxy analogue **2a** and the side chain methylated **2l** retain the full LTB_4 inhibitory activity of phenylbutyryl anthracenone **2**, while their antiproliferative activity is markedly enhanced and comparable to that of the antipsoriatic anthralin. In contrast to anthralin, cytotoxic effects against cell membranes and hydroxyl-radical generation are reduced to a minimum.

6. Experimental protocols

6.1. Chemistry

6.1.1. General

For analytical instruments and methods see Ref. [7].

6.1.2. Preparation of acyl chlorides

Acyl chlorides were prepared from the corresponding carboxylic acids according to the standard literature procedures [20], and the crude products were used in the subsequent acylation steps (Method A).

6.1.3. Preparation of mixed anhydrides

A solution of the appropriate carboxylic acid (10 mmol) and triethylamine (1.01 g, 10 mmol) in absolute ether (25 mL) was cooled on an ice/NaCl bath to 0°C. Ethyl chloroformate (1.09 g, 10 mmol) was added slowly under stirring, and the temperature was kept at 0°C. Then the mixture was warmed to room temperature within 1 h and filtered. The filtrate was washed with a solution of $NaHCO_3$ (0°C) and then with water, dried over $MgSO_4$, and evaporated. The crude products were used in the subsequent acylation steps (Method B).

*6.1.4. General procedure for the acylation of **1** to the 10-substituted 1,8-dihydroxy-9(10H)-anthracenones*

Method A. To a solution of **1** [21] (1.00 g, 4.42 mmol) in absolute toluene (75 mL) and dry pyridine (0.43 mL, 5.50 mmol) was added dropwise a solution of the appropriate acyl chloride (5.50 mmol) in absolute toluene (10 mL) under N_2. The reaction mixture was stirred at room temperature for 12 h, filtered, and the filtrate was evaporated. The residue was purified by column chromatography.

Method B. The appropriate mixed anhydrides (5.50 mmol) in absolute acetone were used in place of the acyl chlorides.

Method C. To a solution of **1** (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) under N_2. The reaction mixture was stirred at room temperature for 4 h, filtered, and the filtrate was evaporated. The residue was purified by column chromatography.

*6.1.4.1. 1,8-Dihydroxy-10-[4-(4-methoxyphenyl)-1-oxobutyl]-9(10H)-anthracenone (**2a**).*

The title compound was obtained from **1** and 4-(4-methoxyphenyl)butyryl chloride [22] according to Method A. Purification by column chromatography using CH_2Cl_2–hexane (19:1) gave a yellow powder; 24% yield; m.p. 116–117°C; FTIR 1711, 1630 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 12.20 (s, 2H), 7.57–6.72 (m, 10H), 5.14 (s, 1H), 3.73 (s, 3H), 2.30–1.90 (m, 4H), 1.70–1.40 (m, 2H); MS $m/z = 402$ (2, M^+), 226 (100). Anal. $C_{25}H_{22}O_5$ (C, H).

*6.1.4.2. 1,8-Dihydroxy-10-[4-(3,4-dimethoxyphenyl)-1-oxobutyl]-9(10H)-anthracenone (**2b**).*

The title compound was obtained from **1** and 4-(3,4-dimethoxyphenyl)butyryl chloride [22] according to Method A. Purification by column chromatography using CH_2Cl_2–ether (49:1) gave a yellow powder; 30% yield; m.p. 117°C; FTIR 1713, 1628 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 12.30 (s, 2H), 7.62–6.32 (m, 9H), 5.20 (s, 1H), 3.83 (s, 3H), 3.78 (s, 3H), 2.32–1.77 (m, 4H), 1.73–1.43 (m, 2H); MS $m/z = 432$ (4, M^+), 207 (100). Anal. $C_{26}H_{24}O_5$ (C, H).

*6.1.4.3. 1,8-Dihydroxy-10-[4-(4-methylphenyl)-1-oxobutyl]-9(10H)-anthracenone (**2c**).*

The title compound was obtained from **1** and 4-tolylbutyric acid according to Method C. Purification by column chromatography using CH_2Cl_2–petroleum ether (8:2) and recrystallisation from benzene–petroleum ether (1:1) gave yellow needles; 13% yield; m.p. 190°C (dec); FTIR 1711, 1632 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 11.93 (s, 2H), 7.66–6.83 (m, 10H), 5.63 (s, 1H), 2.53 (t, $J = 6.9$ Hz, 2H), 2.29 (t, $J = 7.5$ Hz, 2H), 2.23 (s, 3H), 1.55 (tt, $J = 7.5$ Hz, $J = 6.9$ Hz, 2H). Anal. $C_{25}H_{22}O_4$ (C, H).

*6.1.4.4. 1,8-Dihydroxy-10-(1,4-dioxo-4-phenylbutyl)-9(10H)-anthracenone (**2d**).*

The title compound was obtained from **1** and ethyl (4-oxo-4-phenyl)butyrylcarbonate (prepared from 3-benzoylpropionic acid) according to Method B. Purification by column chromatography using CH_2Cl_2–hexane (4:1) gave a yellow powder; 10% yield; m.p. 162–164°C; FTIR 1721, 1694, 1630 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 12.27 (s, 2H), 7.90–6.91 (m, 11H), 5.28 (s, 1H), 3.02 (t, $J = 6$ Hz, 2H), 2.39 (t, $J = 6$ Hz, 2H); MS $m/z = 161$ (100), 105 (31). Anal. $C_{24}H_{18}O_5$ (C, H).

*6.1.4.5. 1,8-Dihydroxy-10-[4-(4-methoxyphenyl)-1,4-dioxobutyl]-9(10H)-anthracenone (**2e**).*

The title compound was obtained from **1** and ethyl [4-(4-methoxyphenyl)-4-oxo]butyrylcarbonate (prepared from 3-(4-methoxybenzoyl)propionic acid [23]) according to Method B. Purification by column chromatography using CH_2Cl_2–hexane (9:1) gave yellow crystals; 6% yield; m.p. 183–185°C; FTIR 1717, 1676, 1628 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 12.30 (s, 2H), 7.90–6.84 (m, 10H), 5.32 (s, 1H), 3.85 (s, 3H), 3.00 (t, $J = 6$ Hz, 2H), 2.40 (t, $J = 6$ Hz, 2H); MS $m/z = 191$ (100), 135 (46). Anal. $C_{25}H_{20}O_6$ (C, H).

*6.1.4.6. (R,S)-1,8-Dihydroxy-10-(1-oxo-3-phenylbutyl)-9(10H)-anthracenone (**2f**).*

The title compound was obtained from **1** and (*R*,*S*)-3-phenylbutyryl chloride [24] according to Method A. Purification by column chromatography using CH_2Cl_2–hexane (7:3) gave a yellow powder; 27% yield; m.p. 146–147°C; FTIR 1711, 1628 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 12.31 (s, 1H), 12.25 (s, 1H), 7.58–6.48 (m, 11H), 5.10 (s, 1H), 3.08–2.98 (m, 1H), 2.28–2.15 (m, 2H), 0.93 (d, $J = 7$ Hz, 3H); MS $m/z = 372$ (8, M^+), 226 (100). Anal. $C_{24}H_{20}O_4$ (C, H).

*6.1.4.7. (R)-1,8-Dihydroxy-10-(1-oxo-3-phenylbutyl)-9(10H)-anthracenone (**2g**).*

The title compound was obtained from **1** and (*R*)-3-phenylbutyryl chloride [25] according to Method A. Purification by column chromatography using CH_2Cl_2–hexane (7:3) gave a yellow powder; 27% yield; m.p. 121–122°C; FTIR 1713, 1630 cm^{-1}; MS $m/z = 372$ (11, M^+), 226 (100). Anal. $C_{24}H_{20}O_4$ (C, H).

*6.1.4.8. (S)-1,8-Dihydroxy-10-(1-oxo-3-phenylbutyl)-9(10H)-anthracenone (**2h**).*

The title compound was obtained from **1** and (*S*)-3-phenylbutyryl chloride [25] according to Method A. Purification by column chromatography using CH_2Cl_2–hexane (7:3) gave a yellow powder; 29% yield; m.p. 121–122°C; FTIR 1713, 1630 cm^{-1}; MS $m/z = 372$ (10, M^+), 226 (100). Anal. $C_{24}H_{20}O_4$ (C, H).

*6.1.4.9. (R,S)-1,8-Dihydroxy-10-[3-(4-methoxyphenyl)-1-oxo-butyl)-9(10H)-anthracenone (**2i**).*

The title compound was obtained from **1** and (*R*,*S*)-3-(4-methoxyphenyl)butyric acid [26] according to Method C. Purification by column chromatography using CH_2Cl_2–petroleum ether (1:1) and recrystallisation from benzene–petroleum ether (8:2) gave yellow crystals; 19% yield; m.p. 125–127°C; FTIR 1705, 1628 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 11.93 (s, 1H), 11.92 (s, 1H), 7.61–6.70 (m, 10H), 5.59 (s, 1H), 3.69 (s, 3H), 3.06–2.77 (m, 3H), 0.95 (d, $J = 6.7$ Hz, 3H); MS $m/z = 402$ (5.6, M^+), 226 (78), 135 (100). Anal. $C_{25}H_{22}O_5$ (C, H).

*6.1.4.10. (R,S)-1,8-Dihydroxy-10-[3-(4-methylphenyl)-1-oxo-butyl)-9(10H)-anthracenone (**2k**).*

The title compound was obtained from **1** and (*R*,*S*)-3-(4-methylphenyl)butyric acid [27] according to Method C. Purification by column chromatography using CH_2Cl_2–/petroleum ether (1:1) and recrystallisation from benzene–petroleum ether (1:1) gave yellow crystals; 6% yield; m.p. 135–137°C; FTIR 1719, 1630 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 11.93 (s, 1H), 11.92 (s, 1H), 7.60–6.91 (m, 10H), 5.59 (s, 1H), 3.06–2.79 (m, 3H), 2.22 (s, 3H), 0.95 (d, $J = 6.7$ Hz, 3H); MS $m/z = 386$ (3.5, M^+), 226 (83), 118 (100). Anal. $C_{25}H_{22}O_4$ (C, H).

*6.1.4.11. (R,S)-1,8-Dihydroxy-10-(1-oxo-4-phenylpentyl)-9(10H)-anthracenone (**2l**).*

The title compound was obtained from **1** and (*R*,*S*)-4-phenylvaleryl chloride (prepared from 4-phenylvaleric acid [28]) according to Method A. Purification by column chromatography using CH_2Cl_2–hexane (3:2) gave a yellow powder; 33% yield; m.p. 82°C; FTIR 1711, 1628 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 12.19 (s, 2H), 7.57–6.76 (m, 11H), 5.12 (s, 1H), 2.55–2.17 (m, 1H), 1.97–1.42 (m, 4H), 1.02 (d, $J = 7$ Hz, 3H); MS $m/z = 386$ (5, M^+), 226 (100). Anal. $C_{25}H_{22}O_4$ (C, H).

*6.1.4.12. (R,S)-1,8-Dihydroxy-10-(1-oxo-3-phenylpentyl)-9(10H)-anthracenone (**2m**).*

The title compound was obtained from **1** and (*R*,*S*)-3-phenylvaleric acid [29] according to Method C. Purification by column chromatography using CH_2Cl_2–petroleum ether (1:1) and recrystallisation from benzene–petroleum ether (7:3) gave yellow needles; 13%

yield; m.p. 107–109°C; FTIR 1705, 1628 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 11.92 (s, 1H), 11.91 (s, 1H), 7.58–6.92 (m, 10H), 5.59 (s, 1H), 2.97 (d, $J = 6.9$ Hz, 2H), 2.78 (tt, $J = 6.9$ Hz, $J = 6.8$ Hz, 1H), 1.36 (dq, $J = 6.8$ Hz, $J = 7.3$ Hz, 2H), 0.56 (t, $J = 7.3$ Hz, 3H); MS $m/z = 386$ (4.0, M^+), 226 (100). Anal. $C_{25}H_{22}O_4$ (C, H).

6.1.5. Analysis by chiral phase chromatography

Enantiomers **2g,h** were analysed by chiral phase HPLC on a Daicel Chiralpak AD 250×4.6 mm^2 column (Daicel, Tokyo, Japan). The isocratic elution conditions were (+)-2-butanol–2-propanol–hexane (1.56:1.17:92.27, v/v/v), flow rate 0.3 mL min^{-1} (Merck-Hitachi L-6200 A), monitored at 220 nm with a Merck L 4000 A UV detector. The retention times for **2g** and **2h** were 40.6 and 37.7 min, respectively.

6.2. Biological assay methods

HaCaT keratinocyte proliferation assay [30], LDH release [5], inhibition of LTB_4 biosynthesis in bovine PMNL [9], and degradation of 2-deoxy-D-ribose [9] were described previously in full detail.

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