# 10-α-Aminoacyl-9(10*H*)-anthracenones: Inhibition of 12(*S*)-HETE Biosynthesis and HaCaT Cell Growth☆

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## Summary

1,8-Dihydroxy-9(10*H*)-anthracenones with a 10-α-aminoacyl group were synthesized using either a mixed-anhydride coupling method or Boc-protected oxazolidinediones. The novel anthracenones were evaluated as inhibitors of the biosynthesis of 12(*S*)-hydroxyeicosatetraenoic acid (12(*S*)-HETE) in epidermal homogenate of mice and for inhibition of the growth of HaCaT keratinocytes. These cells were also tested for their susceptibility for the action of the most potent members of this series on plasma membrane integrity, in order to confirm that inhibition of cell growth is not a result of membrane damage induced by prooxidants released from anthracenones. Hydroxyl-radical generation as measure of the prooxidant potential of the compounds was determined by deoxyribose degradation. The most potent analogues of this series were equally potent as anthralin against 12(*S*)-HETE biosynthesis and keratinocyte proliferation, while oxygen-radical generation and the resulting damage to cell membrane was strongly reduced as compared to the antipsoriatic drug.

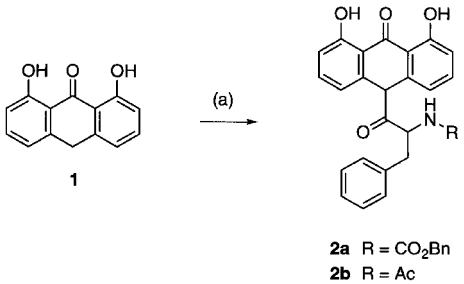
## Introduction

Anthracenones are important agents for the treatment of psoriasis. While antipsoriatic drugs such as vitamin D analogues and retinoids serve only to abate this hyperproliferative skin disorder, drugs such as anthralin (**1**) clear the pathological features of psoriasis totally [1,2]. However, the therapeutic use of this compound is accompanied by severe inflammation of the non-affected skin surrounding the psoriatic lesion [3]. This is a significant drawback which is serious enough to prevent the use of anthralin or reduce patient compliance [4]. Accordingly, the development of a topically effective antipsoriatic anthracenone which should obviate the undesired proinflammatory effects is highly desirable. The mechanism for the inflammatory reactions of the skin associated with anthralin treatment is not completely understood, but several lines of evidence derived from chemical and biological studies have revealed that it is associated with the formation of reactive oxygen species or anthralin radicals and subsequent lipid peroxidation [5,6]. Furthermore, structure–activity relationships of simple analogues of anthralin revealed a marked decrease in antiproliferative activity when any of the 1,8-dihydroxy substituents was modified [7]. Thus, to counteract these proinflammatory mediators, we have designed novel agents combining the required 1,8-dihydroxyanthracenone chromophore and moieties which have a high affinity for iron or/and may act as free radical scavengers, such as catechol-, pyrogallol- or hydroxamate-terminated side chains [8,9].

In order to broaden the structural diversity within our collection of antipsoriatic anthracenones with modulated release of oxygen radicals, we sought alternatives to anthralin which retain potent inhibitory action against keratinocyte growth but do not induce damage to the cell membrane. The work presented here describes the synthesis and evaluation of new 10-α-aminoacyl-1,8-dihydroxy-9(10*H*)-anthracenones such as **2a**.

## Chemistry

The conventional introduction of a 10-acyl substituent onto anthracenones is achieved by reaction of acyl chlorides with **1** in the presence of pyridine [10]. However, this method is not efficient for the preparation of the desired 10-α-aminoacyl substituted anthracenones, since reaction of **1** with an *N*-Cbz-protected amino acid chloride, *N*-benzyloxycarbonyl-L-phenylalanoyl chloride [11], gave only a poor yield of **2a** (Scheme 1). Carboxylic acids may also be directly attached to the 10-position of **1** in the presence of the coupling agent dicyclohexylcarbodiimide (DCC) [12]. However, treatment of **1** with *N*-acetyl-L-phenylalanine under these conditions gave only trace amounts of **2b**, which was obtained together with unidentified byproducts and proved difficult to purify. A somewhat more promising route to this compound appeared to be via a standard mixed-anhydride coupling method [13].




**Scheme 1.** Reagents: (a) *N*-Cbz-L-Phe chloride, toluene, pyridine (R = $CO_2Bn$); *N*-Ac-L-Phe, ethyl chloroformate, $NEt_3$, 0 °C; acetone, pyridine (R = Ac).

**Abbreviations:**
Cbz, benzyloxycarbonyl; DCC, dicyclohexylcarbodiimide; DMSO, dimethyl sulfoxide; LDH, lactate dehydrogenase; 12(*S*)-HETE, 12(*S*)-hydroxyeicosatetraenoic acid; 12-LO, 12-lipoxygenase; MDA, malondialdehyde.

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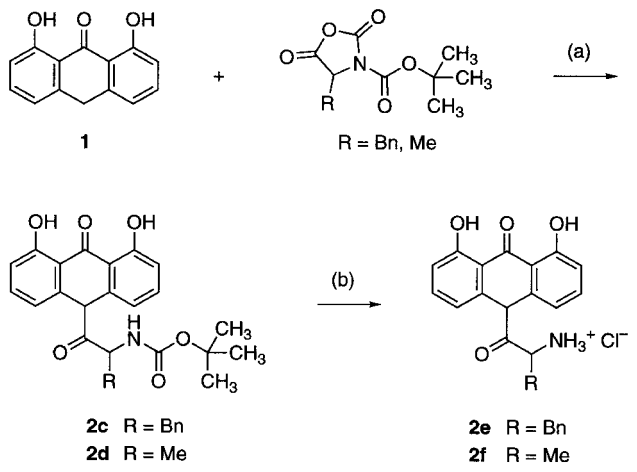



**Scheme 2.** Reagents: (a) $NEt_3$, acetone, $N_2$; (b) ethyl acetate, 36% HCl, $N_2$.

Thus, a pure sample of **2b** could be isolated from the reaction of **1** in acetone with the mixed-anhydride of *N*-acetyl-L-phenylalanine and ethyl chloroformate (Scheme 1).

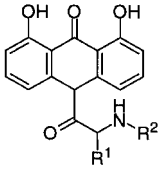
A much more satisfactory route is illustrated in Scheme 2. By use of the Leuchs-type of mixed anhydrides, i.e. the requisite Boc-protected oxazolidinediones, with **1** in the presence of triethylamine in acetone we were able to obtain the 10-α-aminoacyl anthracenones **2c** and **2d**, which upon cleavage with ethyl acetate and hydrochloric acid provided analogues **2e** and **2f**, respectively, isolated as their hydrochlorides.

## Biological Evaluation

Psoriasis is a widespread, inflammatory and scaling skin disease, which is characterized by increased cell proliferation of the epidermis [14]. Both features of psoriasis, hyperproliferation and inflammation, are resolved following topical therapy with anthralin. Inhibition of the biosynthesis of lipoxygenase-derived metabolites may be appropriate to manage the inflammatory component of the disease [15]. 12-Hydroxyeicosatetraenoic acid (12-HETE) is the major lipoxygenase (LO) product found in the skin [16] and has been demonstrated to be present in high levels in psoriatic epidermis [17]. Furthermore, the characterization of LO activity in human epidermis indicates that germinal layer keratinocytes contain a highly active 12-LO which is selectively expressed at a higher level during psoriatic inflammation [18]. 12-HETE stimulates keratinocyte proliferation and induces histological changes characteristic of psoriasis [19–21]. Based on this information, we evaluated the ability of the novel compounds to inhibit the 12-LO pathway in epidermal homogenate of mice [22].

Moreover, the biological activity of drugs useful as antipsoriatic agents may be evaluated by their antiproliferative activity in cell cultures [23], which may be critical in resolving hyperproliferation of psoriasis. HaCaT keratinocytes can be used as a model for highly proliferative epidermis, and this

**Table 1.** Inhibition of 12(*S*)-HETE biosynthesis in mouse epidermis, antiproliferative activity and cytotoxicity against HaCaT Cells, and hydroxyl-radical generation of 10-α-aminoacyl-1,8-dihydroxy-9(10*H*)-anthracenones.




| Compd | $R^1$ | $R^2$ | 12-LO $IC_{50}$ (μM)[a] | AA $IC_{50}$ (μM)[b] | LDH (mU)[c] | •OH[d] |
|---|---|---|---|---|---|---|
| **1** | anthralin | | 9 | 0.7 | 294 | 2.89 ± 0.14 |
| **2a** | Bn | $CO_2$Bn | 6 | 0.8 | 147[e] | 0.49 ± 0.09 |
| **2b** | Bn | Ac | 30 | 1.2 | ND | 1.20 ± 0.26 |
| **2c** | Bn | $CO_2$*tert*-Bu | 10 | 2.1 | ND | 0.52 ± 0.13 |
| **2d** | Me | $CO_2$*tert*-Bu | 20 | 3.9 | ND | 0.47 ± 0.10 |
| **2e** | Bn | H | 30 (58) | 0.7 | 152[e] | 2.32 ± 0.19 |
| **2f** | Me | H | 30 (52) | 0.8 | 180 | 3.05 ± 0.39 |

[a] Inhibition of 12(*S*)-HETE biosynthesis in mouse epidermal homogenates. Inhibition was significantly different with respect to that of the control ($N = 3$ or more, $P$ 0.01). Values in parentheses are percent inhibition at the indicated concentrations (μM), and standard errors average 10% of the indicated values. [b] Antiproliferative activity against HaCaT cells. Inhibition of cell growth was significantly different with respect to that of the control ($N = 3$, $P < 0.05$). [c] 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%, $P < 0.01$). [d] Deoxyribose degradation as a measure of hydroxyl-radical generation. Indicated values are μmol of malondialdehyde/mmol of deoxyribose released by 75 μM test compound (controls < 0.1). Values are significantly different with respect to control; $P < 0.01$. [e] Values are not significantly different with respect to vehicle control; $P < 0.01$. ND = not determined.

nontransformed human cell line was described as an extremely sensitive target for the antiproliferative action of anthralin [24]. Accordingly, 10-α-aminoacyl anthracenones were also evaluated for inhibition of the growth of HaCaT cells. Moreover, keratinocytes were tested for their susceptibility for the action of the most potent members of this series on plasma membrane integrity, in order to confirm that inhibition of cell growth is not a result of membrane damage induced by prooxidants released from anthracenones. Finally, the prooxidant potential of the compounds was determined by deoxyribose degradation.

## Results and Discussion

### *Hydroxyl-Radical Generation*

The deoxyribose assay is a sensitive test for the production of hydroxyl radicals [25]. The release of malondialdehyde (MDA) is a measure of this feature and reflects the prooxidant properties of the compounds. Table 1 shows that substitution at C-10 of anthralin with *N*-acylated α-aminoacyl groups strongly reduces the intensity of hydroxyl-radical generation (**2a**, **2c**, **2d**). However, analogues without amino protected group such as **2e** and **2f** are oxygen-radical generators with an intensity comparable to that of anthralin. Also, elevated amounts of deoxyribose damage are observed for the *N*-acetylated analogue **2b**.

### *Inhibition of 12(S)-HETE Biosynthesis*

In this study we used epidermal strips from mouse skin to investigate the effects of the novel analogues. To exclude oxidation of arachidonic acid by a nonspecific free radical mechanism which affords racemic 12-HETE, the absolute stereochemistry of 12-HETE was determined by chiral phase HPLC to confirm the presence of the *S*-enantiomer [22].

Except for analogues **2a** and **2c**, introduction of an α-aminoacyl substituent into the anthracenone nucleus seems to decrease the inhibitory activity against the epidermal 12-LO enzyme as compared to anthralin.

### *Antiproliferative Activity*

The novel analogues were also evaluated in whole cell studies. Their antiproliferative potential was determined directly by counting the keratinocytes. The cells were enumerated in Neubauer counting chambers by phase contrast microscopy after 48 h of treatment. Shown in Table 1 is the ability of all compounds to inhibit the proliferation of HaCaT cells as demonstrated by reduction in cell number over time as compared to control plates.

In general, all novel analogues exhibit potent antiproliferative activity. Compounds **2e** and **2f**, derived from phenylalanine and alanine, respectively, and each possessing an unsubstituted amino group, show equivalent potency. Compound **2a** is also equipotent to these analogues, regardless of the *N*-benzyloxycarbonyl substituent, whereas the *N*-acetyl analogue **2b** is a somewhat weaker inhibitor of keratinocyte growth. However, *N*-protected compounds **2c** and **2d** with the bulky *tert*-butyl ester moiety are less potent than their unprotected congeners **2e** and **2f**, respectively. Furthermore, it should be noted that the antiproliferative activity of anthracenones seems to be independent of their ability to inhibit the biosynthesis of 12(*S*)-HETE, which was found to be a stimulator of epidermal proliferation *in vivo* [19] and to be mitogenic in endothelial cells [26]. Moreover, analogue **2a** which is a much weaker generator of hydroxyl radicals than anthralin documents that potency comparable to that of the antipsoriatic drug is not directly related to the hydroxyl-radical generating capability of the compounds. However, compounds **1**, **2e**, and **2f** reveal that the greatest potency ($IC_{50}$ 0.7–0.8 μM) is coincident with the strongest prooxidant activity.

### *Integrity of the Plasma Membrane*

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. Anthralin has been reported to cause membrane damage, but did not directly lead to substantial membrane destruction [24]. Cytotoxicity against HaCaT keratinocytes by the most potent cell growth inhibitors was assessed by the activity of lactate dehydrogenase (LDH) released into the culture medium, which is commonly used as indicator of plasma membrane damage. In this assay, LDH release by anthralin significantly exceeded that of the vehicle control. On the other hand, the activity of the novel analogues was due to cytostatic rather than cytotoxic effects, as LDH release was unchanged as compared to controls. The exception was the potent hydroxyl-radical generator **2f**, which shows slightly increased membrane damage as compared to control cultures. By contrast, cytotoxic effects of **2a** and even for **2b**, the latter of which is also able to produce appreciable amounts of hydroxyl radicals, were not apparent at 2 μM.

### *Stability*

Anthralin staining is an important limitation of its therapeutic use and is primarily due to its oxidation to the anthralin radical which dimerizes or polymerizes to give inactive products such as bianthrone and the so-called anthralin-brown, respectively [27]. With the 10-α-aminoacyl side chain, this side effect should be reduced because of steric hindrance to radical dimerization. To prove this hypothesis, we have determined the decomposition of the *N*-Cbz protected **2c** and the *N*-unprotected **2e** in DMSO and ethanol by RP-HPLC over a 5-day period at 25 °C. Table 2 shows the half-lives of the selected compounds together with those of anthralin in

**Table 2.** Half-lives ($t_{1/2}$, h) and degradation products of selected 10-α-aminoacyl-substituted 1,8-dihydroxy-9(10*H*)-anthracenones in DMSO and ethanol at 25 °C.

| Cpd | $t_{1/2}$ (h) DMSO | $t_{1/2}$ (h) ethanol | Degradation products detected by HPLC |
|---|---|---|---|
| **2c** | 51 | 8 | dantron |
| **2e** | 0.5 | 0.6 | anthralin, anthralin dimer, polymers |
| **anthralin** (**1**) | 18 | 3 | dantron, anthralin dimer, polymers |

the corresponding solvents. Our finding is that *N*-protected **2c** is considerably more stable than anthralin, whereas deprotection (**2e**) dramatically decreased stability. For **2c** and anthralin, decomposition was more favored in the protic solvent ethanol than in the aprotic DMSO while **2e** was also slightly faster degraded in DMSO. Moreover, decomposition of **2c** resulted in formation of the corresponding anthracenedione, dantron, as also observed with other 10-acylated anthracenones [28]. However, degradation products of **2e** are anthralin and the anthralin dimer, precursors of anthralin-brown, which is somewhat unfortunate.

## Conclusions

We have synthesized a small series of 10-α-aminoacyl-1,8-dihydroxy-9(10*H*)-anthracenones and have determined their biological activity in various assays. Appendage of an amino acid group to anthralin results in an interesting *in vitro* profile. Based on this limited data set, we found that the ability of the compounds to inhibit 12(*S*)-HETE biosynthesis is quite variable in this series, whereas their capacity to inhibit the growth of keratinocytes is consistently high. With respect to both of these features, analogue **2a** is equipotent to anthralin and compares favorably in further tests with this antipsoriatic drug. In particular, hydroxyl-radical generation by **2a** is largely reduced and damage to cell membrane was not observed at concentrations necessary for potent antiproliferative action. However, the observation that an α-aminoacyl substituent at the 10-position of the molecules only slightly increases the stability as compared to anthralin is somewhat disappointing.

## Experimental Part

Melting points: Büchi 510 melting point apparatus (uncorrected). $^1$H-NMR: Varian EM 390 (90 MHz) or Bruker Spectrospin WM 250 spectrometer (250 MHz). Fourier-transform IR spectra (KBr): Nicolet 510M FTIR spectrometer. UV spectra: Kontron 810 spectrometer. Mass spectra (EI, unless otherwise stated): Varian MAT CH5 spectrometer (70 eV). HPLC: Kontron 420, 735 LC UV detector, 250- × 4-mm column (4- × 4-mm precolumn), LiChrospher 100 RP18 (5-µm particles; Merck, Darmstadt, Germany). Data recording and analysis: MacLab data acquisition system (WissTech, Germany), software Peaks (Apple Macintosh computer). Chromatography refers to column chromatography on silica gel (E. Merck, 70–230 mesh).

*10-(2-Benzyloxycarbonylamino-1-oxo-3-phenylpropyl)-1,8-dihydroxy-9(10H)-anthracenone (***2a***)*

To a solution of **1** [29] (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 *N*-benzyloxycarbonyl-L-phenylalanoyl chloride [11] (1.55 g, 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 chromatography ($CH_2Cl_2$) to afford **2a** as a yellow powder (10%): mp 152–154 °C; $^1$H-NMR ($CDCl_3$) δ 12.36 (s, 2H, OH), 7.61–6.62 (m, 16H, Ar), 5.34 (s, 1H, 10-H), 4.89–4.56 (m, 4H, CHNH, $OCH_2$), 2.86–2.66 (m, 2H, C*H*$_2$CH); FTIR 3332 (NH), 1705 (CO), 1674 (OCONH), 1630 cm$^{-1}$ (CO···HO). Anal. ($C_{31}H_{25}NO_6$) C, H, N.

*10-(2-Acetylamino-1-oxo-3-phenylpropyl)-1,8-dihydroxy-9(10H)-anthracenone (***2b***)*

Mixed-anhydride coupling method:[13] A solution of *N*-acetyl-L-phenylalanine (2.07 g, 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 *N*-acetyl-L-phenylalanoylethylcarbonate (1.53 g, 5.50 mmol) in absolute acetone (10 mL) was used in the subsequent acylation of **1** in acetone (48 h, room temperature), as described for **2a**. Purification by chromatography ($CH_2Cl_2$/$Et_2O$ 9/1) afforded **2b** as yellow crystals (10%): mp 162–164 °C; $^1$H-NMR (250 MHz, $CDCl_3$) δ 12.63, 12.15 (2 × s, 2H, OH), 7.68–6.74 (m, 11H, Ar), 6.18, 5.43 (2 × d, $J$ = 8 Hz, 1H, NH), 5.37, 4.39 (2 × s, 1H, 10-H), 5.33–5.25, 5.11–5.02 (2 × m, 1H, CH), 3.57–3.49, 2.91–2.82 (2 × q, $J_{AB}$ = 14 Hz, $J_{BX}$ = 7 Hz, 1H, *H*CH), 3.40–3.31, 2.74–2.65 (2 × q, $J_{AB}$ = 14 Hz, $J_{AX}$ = 7 Hz, 1H, HC*H*), 2.17, 1.78 (2 × s, 3H, Me); FTIR 3363 (NH), 1763 (CON), 1717 (CO), 1630 cm$^{-1}$ (CO···HO). Anal. ($C_{25}H_{21}NO_5$) C, H, N.

*1,8-Dihydroxy-10-[2-(1,1-dimethylethoxycarbonylamino)-1-oxo-3-phenylpropyl]-9(10H)-anthracenone (***2c***)*

To a solution of **1** (1.00 g, 4.42 mmol) in absolute acetone (160 mL) under $N_2$ were added triethylamine (0.66 mL, 4.75 mmol) and Boc-L-phenylalanine-*N*-carboxyanhydride (3.81 g, 12 mmol, Fluka), and the mixture was stirred for 12 h under $N_2$. Then the mixture was concentrated, and the residue was purified by chromatography ($CH_2Cl_2$) to afford **2c** as a yellow powder (44%): mp 147–149 °C; $^1$H-NMR ($CDCl_3$) δ 12.27 (s, 2H, OH), 7.50–6.71 (m, 11H, Ar), 5.38 (s, 1H, 10-H), 4.73–4.33 (m, 2H, CHNH), 2.87 (m, 2H, $CH_2$), 1.29 (s, 9H, *tert*-Bu); FTIR 3349 (NH), 1726 (CO), 1696 (OCONH), 1630 cm$^{-1}$ (CO···HO). MS *m/z* 400 (2) (M - $^{\bullet}$O*tert*-Bu)$^+$, 226 (100). Anal. ($C_{28}H_{27}NO_6$) C, H, N.

*1,8-Dihydroxy-10-[2-(1,1-dimethylethoxycarbonylamino)-1-oxopropyl]-9(10H)-anthracenone (***2d***)*

**2d** was prepared from Boc-L-alanine-*N*-carboxyanhydride as described for **2c**. Purification by chromatography ($CH_2Cl_2$/$Et_2O$ 95/5) afforded **2d** as a yellow powder (25%): mp 150–151 °C; $^1$H-NMR ($CDCl_3$) δ 12.24 (s, 2H, OH), 7.63–6.88 (m, 6H, Ar), 5.47 (s, 1H, 10-H), 4.83–4.40 (m, 2H, CHNH), 1.39 (s, 9H, *tert*-Bu), 1.09 (d, $J$ = 7 Hz, 3H, Me); FTIR 3363 (NH), 1725 (CO), 1674 (OCONH), 1632 cm$^{-1}$ (CO···HO). PI-FDMS *m/z* 397 ($M^{+\bullet}$). Anal. ($C_{22}H_{23}NO_6$) C, H, N.

*10-(2-Amino-1-oxo-3-phenylpropyl)-1,8-dihydroxy-9(10H)-anthracenone Hydrochloride (***2e***)*

To a solution of **2c** (0.10 g, 0.21 mmol) in ethyl acetate (8 mL) was added, dropwise over 30 min, 36% HCl (1 mL). The mixture was stirred until the reaction was completed (TLC control). Then the solvent was evaporated, and the residue was digested with small amounts of ether and filtered by suction to afford **2e** as a yellow powder (81%): mp 191–192 °C (dec); $^1$H-NMR ($CD_3OD$) δ 7.65–6.97 (m, 11H, Ar), 4.76 (q, $J_{AX}$ = 9.5 Hz, $J_{MX}$ = 5 Hz, 1H, CH), 3.56 (q, $J_{AM}$ = 14.5 Hz, $J_{MX}$ = 5 Hz, 1H, *H*CH), 2.86 (q, $J_{AM}$ = 14.5 Hz, $J_{AX}$ = 9.5 Hz, 1H, HC*H*), 10-H, 1,8-$(OH)_2$, $NH_2$ exchanged; FTIR 2855 ($NH_3^+$), 1721 (CO), 1630 cm$^{-1}$ (CO···HO). PI-LISIMS (DMSO, glycerol) *m/z* 452 (M+H$^+$+DMSO), 374 M+H$^+$). Anal. ($C_{23}H_{20}ClNO_4$) C, H, N.

*(2-Amino-1-oxopropyl)-1,8-dihydroxy-10-9(10H)-anthracenone Hydrochloride (***2f***)*

**2f** was prepared by deprotection of **2d** as described for **2e**. Recrystallization from acetone afforded **2f** as yellow crystals (76%): mp 193–194 °C (dec); $^1$H-NMR (250 MHz, $CD_3OD$) δ 7.63–6.97 (m, 6H, Ar), 4.54 (q, $J$ = 7 Hz, 1H, CH), 1.57 (d, $J$ = 7 Hz, 3H, Me), 10-H, 1,8-$(OH)_2$, $NH_2$ exchanged; FTIR 2925 ($NH_3^+$), 1725 (CO), 1630 cm$^{-1}$ (CO···HO). PI-LISIMS (DMSO, glycerol) *m/z* 390 (M+H$^+$+glycerol), 298 (M+H$^+$). Anal. ($C_{17}H_{16}ClNO_4$) C, H, N.

*Degradation of 2-Deoxy-D-ribose*

The deoxyribose assay was conducted as described [30]. The reaction mixtures contained the following reagents at the final concentrations stated: 0.3 mL of $KH_2PO_4$-KOH buffer, pH 7.4 (30 mM), 0.2 mL of $H_2O$ (double distilled), 0.2 mL of 2-deoxy-D-ribose (2 mM), 0.2 mL of $FeCl_3$•$6H_2O$

(0.1 mM), and 0.1 mL of anthracenone derivative (75 μM). After incubation for 2 h at 37 °C in a shaking water bath, TBA-reactive material was measured at 532 nm.

*Inhibition of 12(S)-HETE Biosynthesis*

Preparation of the epidermal homogenate from NMRI-mice and the epidermal 12-LO assay were performed as described [22]. Test compounds were preincubated for 5 min at 37 °C, and the concentration of 12(*S*)-HETE formed after 10 min was measured by reversed-phase HPLC analysis. 12(*S*)-HETE was analyzed by chiral phase chromatography as described [22].

*Cell Culture and Determination of Cell Growth*

HaCaT cells [31] were cultivated and the cell proliferation assay was performed as previously described [8]. After 48 h of incubation, cell growth was determined by enumerating the dispersed cells by phase contrast microscopy. Inhibition was calculated by the comparison of the mean values of the test compound ($N$ = 3) with the control ($N$ = 6–8) activity: (1 - test compound/control) × 100. Inhibition was statistically significant compared to that of the control (Student's t-test; $P < 0.05$). Each $IC_{50}$ value was derived by interpolation of a log inhibitor concentration versus response plot using four or more concentrations of the compound, spanning the 50% inhibition point.

*Lactate Dehydrogenase Release*[24]

HaCaT cells were incubated with the test compounds (2 μM) for 4 h at 37 °C. Extracellular LDH activity was measured using the UV method with pyruvate and NADH and is expressed in mU/mL. Appropriate controls with the vehicle were performed ($P < 0.01$; $N$ = 3, SD < 10%).

*Stability Studies*[28]

The test compounds (1 mM) were dissolved in the pertinent solvents (p.a.) and were kept in the dark at room temperature. Aliquots of 20 μL were withdrawn after appropriate time and subjected to HPLC analysis. Eluant: methanol/water/acetic acid (77/23/0.1, adjusted to pH 5.5 with $NH_3$); flow rate 1 mL/min; 200 bar; detected at 254 nm.

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☆ Dedicated to Prof. Dr. B. Unterhalt, Münster, on the occasion of his 65th birthday.

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