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**Short communication**

# 10-Aminomethylene-1,8-dihydroxy-9(10*H*)-anthracenones: Inhibitory action against 5-lipoxygenase and the growth of HaCaT cells

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**Summary** — A novel series of 10-aminomethylene substituted 1,8-dihydroxy-9(10*H*)-anthracenones was synthesized and evaluated both in the bovine polymorphonuclear leukocyte 5-lipoxygenase (5-LO) and in the HaCaT keratinocyte proliferation assay for their enzyme inhibitory and antiproliferative activity, respectively. The synthesis required readily available formanilides as starting materials and a modified Vilsmeier type reaction with the parent anthracenone. The most potent inhibitor of 5-LO was a 4-hydroxyphenyl analog, whereas a 4-nitrophenyl substituent was essential for potent antiproliferative activity. The results of this study indicate that an activated double bond at C-10 of phenylalkylidene-substituted anthracenones is required for potency.

**anthracenone / antiproliferative activity / HaCaT / 5-lipoxygenase / Vilsmeier**

## Introduction

Psoriasis is a chronic inflammatory skin disease characterized by hyperproliferation of epidermal keratinocytes. Among agents with topical antipsoriatic activity, anthracenones such as anthralin **1** are widely used because of their reliability and effectiveness. However, their clinical efficacy is limited by side effects such as inflammation of the skin surrounding treated psoriatic plaques [1]. Several lines of evidence derived from chemical and biological studies have revealed that the mechanism of anthracenone-induced skin inflammation is associated with the formation of reactive oxygen species or anthracenone radicals [2, 3].

As part of a program to develop improved topical antipsoriatic agents, we have synthesized and tested a number of 1,8-dihydroxy-9(10*H*)-anthracenones substituted at various positions of the anthracenone nucleus [4–6]. As a result, analogs in which one or both active methylene protons at C-10 of the anthracenone were replaced by suitable substituents showed

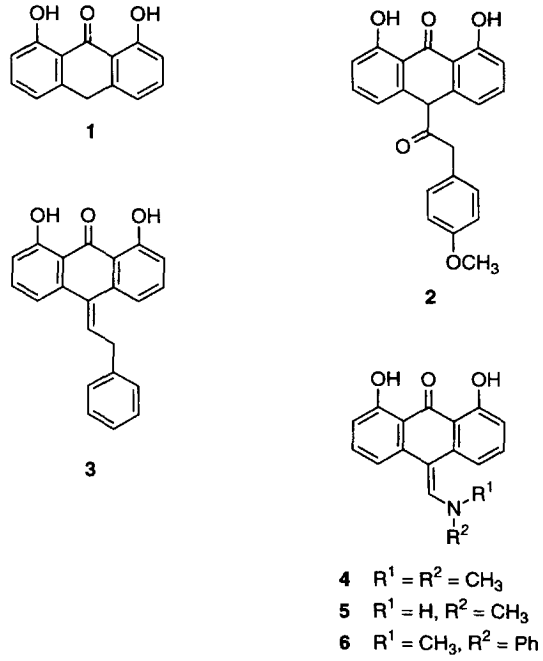



**Fig 1.**

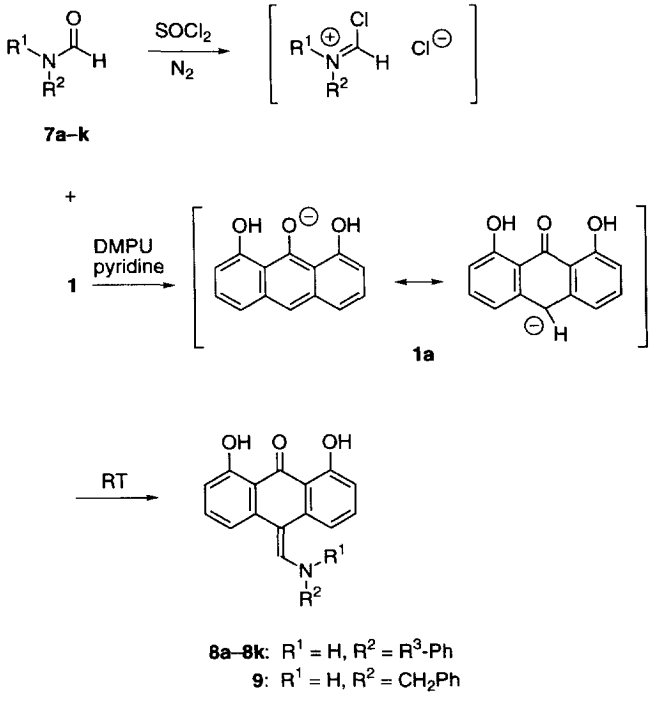
*Correspondence and reprints
*Abbreviations:* DMPU, 1,3-Dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone; 5-HETE, 5-hydroxyeicosatetraenoic acid; $LTB_4$, leukotriene $B_4$; 5-LO, 5-lipoxygenase; PMNL, polymorphonuclear leukocytes.

substantially reduced release of reactive oxygen species [4]. Of these series, compound **2** is currently undergoing clinical evaluation as a topical treatment for psoriasis.

As with the 10-phenylacyl substituted anthracenones, also 10-phenylalkylidene substituted analogs such as **3** were found to be inhibitors of 5-LO and the growth of human keratinocytes, but suffered from nonspecific toxic effects. This is thought to be related to nucleophilic attack by thiol-containing biomolecules at the double bond of the 10-substituent, which is activated by the conjugated keto group at C-9 of the anthracenone, to give Michael addition thioethers.

In an attempt to broaden our understanding of the mechanism of antiproliferative action of the 10-phenylalkylidene anthracenones, we decided to replace the 10-phenylalkylidene substituent with a 10-aminomethylene substituent, distinguished by the incorporation of a nitrogen atom into the alkylidene chain. This substituent would be unlikely to undergo Michael type reactions, because of the electron-donating nitrogen adjacent to the double bond of the alkylidene chain.

We have prepared a smaller series of 10-*N*-aryl- and some 10-*N*-alkylaminomethylene substituted anthracenones to address the above discussed problem. In this paper, we describe the synthesis and the activity of this class of compounds against 5-LO and the growth of HaCaT cells.




**Scheme 1.**

## Chemistry

We have recently shown that reaction of benzoyl chloride with anthralin **1** in DMF results in the formation of the 10-aminomethylene substituted anthracenone **4** [7]. As anthralin anion **1a** (scheme 1) is the predominant form in basic solutions and in DMF [8–10], the formation of **4** was rationalized as a modified Vilsmeier reaction involving electrophilic substitution at the 10-position of the quite electron-rich, nucleophilic polyhydroxyanthracene anion **1a** [7]. In this case, the disubstituted formamide was activated by benzoyl chloride, in place of the more common $POCl_3$. We have used this finding to develop a method for rapid preparation of the desired 10-aminomethylene-1,8-dihydroxy-9(10*H*)-anthracenones. However, using *N*-methylformamide or *N*-methylformanilide in place of DMF gave only poor yields of the anthracenone **5** and even failed to produce **6**, respectively. When $POCl_3$ was used as the activating species, the yields were only slightly better than those obtained with benzoyl chloride.

Scheme 1 shows the synthesis of anthracenones **8a–k** and **9**. The starting formanilides **7a–k** were prepared by established procedures from the corresponding anilines [11]. In the subsequent Vilsmeier reaction, DMPU together with small amounts of pyridine were used as a solvent to convert **1** into the required nucleophile **1a**. Activation of the formanilides was achieved under modified Vilsmeier conditions using $SOCl_2$, and the reaction was performed at room temperature to avoid thermal α-elimination to give isonitriles [12]. Phenol **8l** was prepared by deprotection of the corresponding methyl ether **8g** with boron tribromide.

## Pharmacology

Lipoxygenase products are lipid mediators with an important role in inflammatory disease states such as psoriasis [13]. The regulation of the abnormal arachidonic acid metabolism has become an attractive target for pharmacological intervention in psoriasis [14], since increased concentrations of leukotrienes and hydroperoxyeicosatetraenoic acids (HETEs) were measured in psoriatic skin [15]. As a potentially viable

approach to antipsoriatic therapy, 5-LO inhibitory activity was determined by measuring production of leukotriene $B_4$ ($LTB_4$) and 5-HETE in bovine polymorphonuclear leukocytes (PMNL).

However, psoriasis involves both inflammatory and hyperproliferative processes [16]. Besides leukocyte infiltration one major abnormality is the excessive growth of keratinocytes in the epidermis. Therefore, antipsoriatic compounds have to be targeted towards both components of the disease [16]. In vitro cultured cell systems are powerful tools in the identification of potential antipsoriatic agents having antiproliferative activity [17].

The antiproliferative potential of the compounds was studied by evaluating their ability to inhibit the proliferation of HaCaT cells [18], a rapidly multiplying human keratinocyte cell line. Proliferation of the keratinocytes was determined directly by counting the dispersed cells under a phase-contrast microscope after 48 h of treatment.

## Biological results and discussion

Anthralin itself is only a moderate 5-LO inhibitor. In isolated bovine PMNL it inhibited the production of $LTB_4$ with an $IC_{50}$ of 37 μM [4]. As shown in table I, we found that many compounds of the new 10-aminomethylene-substituted series showed improved activity as compared to **1**. The most active compound of this series is the phenol **8l**, which is about a 100-fold more potent than anthralin in this test. The mechanistic rationale for this compound, which is also proposed for other phenolic compounds, is reduction of ferric to ferrous enzyme [19], as 5-LO contains a nonheme iron that is converted to the ferric form upon activation [20]. In contrast to previous work [5, 21], no straightforward correlation between 5-LO inhibition and lipophilicity of the compounds (log *P* values are given in table I) was observed, even though high lipophilicity seems to be detrimental to activity, eg, **6**, **8d**, and **8h**.

The compounds in table I were also tested for antiproliferative effects as demonstrated by reduction in cell number over time as compared to control plates. In general, the 10-aminomethylene substituted analogs are weaker inhibitors of the growth of HaCaT cells than anthralin, with the exception of the nitro derivative **8e**. $LTB_4$ can affect keratinocyte proliferation [22, 23] and cultured HaCaT keratinocytes express the 5-LO gene [24]. The keratinocyte 5-LO pathway may therefore play a primary role in skin biology. However, the negative results obtained with **8e** in the 5-LO assay suggests a different mode of action for the strong antiproliferative activity of compound **8e**.

Rather, the results may be interpreted in terms of the susceptibility of the compounds towards Michael-type reactions with critical biomolecules. Accordingly, an activated double bond at C-10 of the anthracenone appears to be an important structural requirement. In support of this, **8e** is a potent inhibitor of cell growth, as the nitrophenyl substituent is able to delocalize the electrons of the amino nitrogen, which is responsible for deactivation of the double bond of the alkylidene chain.

In conclusion, our in vitro findings offer a mechanistic rationale as to why the 10-phenylalkylidene substituted anthracenones are in general more potent inhibitors of cell growth than their aminomethylene congeners described in this paper. This suggests that in order to reduce nonspecific toxicity of anthracenone derivatives, an effective replacement of the 10-phenylalkylidene substituent with a 10-aminomethylene substituent is not possible without significantly sacrificing potency.

## Experimental protocols

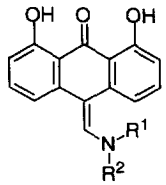
Melting points were determined with a Büchi 510 melting point apparatus and are uncorrected. Chromatography refers to column chromatography on silica gel (Merck, 70–230 mesh). $^1$H-NMR spectra were recorded with a Bruker Spectrospin WM 250 spectrometer (250 MHz), using tetramethylsilane as an internal standard. Fourier-transform IR spectra (KBr) were recorded on a Nicolet 510M FTIR spectrometer. Mass spectra (EI, unless otherwise stated) 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 x 4 mm column (4 x 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.

*1,8-Dihydroxy-10-[(N,N-dimethylamino)methylene]-9(10H)-anthracenone **4** [7]*

*1,8-Dihydroxy-10-[(N-methylamino)methylene]-9(10H)-anthracenone **5***

To a solution of **1** (1.50 g, 6.63 mmol) in *N*-methylformamide (10 mL) was added $POCl_3$ (0.93 g, 6.63 mmol) in one portion under $N_2$, and the solution was stirred until the reaction was complete (TLC control). Then the mixture was poured into ice–water (100 mL), the resulting precipitate was collected, washed with ice–water (3 x 20 mL), and filtered by suction. The product was dissolved in $CH_2Cl_2$ (100 mL), dried over $Na_2SO_4$, and the solvent was evaporated. Purification by column chromatography using $CH_2Cl_2$ afforded light-red crystals: 25% yield; mp 226–228 °C; FTIR 3390, 1627 cm$^{-1}$; $^1$H-NMR ($CDCl_3$) δ 13.26 (s, 1H), 12.95 (s, 1H), 7.57–6.70 (m, 6H), 7.41 (d, 1H, $J$ = 8.5 Hz), 6.19 (d, 1H, exchangeable with $D_2O$, $J$ = 8.5 Hz), 3.17 (s, 3H); MS *m/z* = 276 (100, M$^+$). Anal $C_{16}H_{13}NO_3$ (C, H, N).

**Table I.** 5-LO inhibition in bovine PMNL and antiproliferative activity against HaCaT cells by 10-aminomethylene-1,8-dihydroxy-9(10*H*)-anthracenones:




| *Compound* | *R¹* | *R²* | *Log P* | *5-LO $IC_{50}$ (μM)*[a] | *AA $IC_{50}$ (μM)*[b] |
|---|---|---|---|---|---|
| **4** | $CH_3$ | $CH_3$ | 3.18 | 21 | 2.1 |
| **5** | H | $CH_3$ | 3.55 | 2 | 4.6 |
| **6** | $CH_3$ | Ph | 5.07 | > 30 | 5 |
| **8a** | H | Ph | 4.96 | 13 | > 5 |
| **8b** | H | 4-F-Ph | 4.64 | 5 | 5 |
| **8c** | H | 4-Cl-Ph | 5.06 | 16 | 5 |
| **8d** | H | 4-Br-Ph | 5.19 | > 30 | > 5 |
| **8e** | H | 4-$NO_2$-Ph | 4.70 | > 30 | 0.8 |
| **8f** | H | 2-$CH_3O$Ph | 4.37 | > 30 | > 5 |
| **8g** | H | 4-$CH_3O$Ph | 4.90 | 25 | > 5 |
| **8h** | H | 2,4-$(CH_3O)_2$Ph | 5.51 | > 30 | 5 |
| **8i** | H | 3,4-$(CH_3O)_2$Ph | 4.37 | > 30 | > 5 |
| **8k** | H | 3,4-$OCH_2O$Ph | 4.74 | 30 | > 5 |
| **8l** | H | 4-OH-Ph | 4.01 | 0.3 | > 5 |
| **9** | H | $PhCH_2$ | 4.23 | 24 | 2.6 |
| **1** | anthralin | | 4.32 | 37 | 0.6 |

[a]Inhibition of 5-HETE and $LTB_4$ 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 the standard inhibitor for 5-LO ($IC_{50}$ = 0.4 μM). [b]Antiproliferative activity against HaCaT cells. Inhibition of cell growth was significantly different with respect to that of the control, $N = 3$, $P < 0.01$.

*1,8-Dihydroxy-10-[(N-methyl-N-phenylamino)methylene]-9(10H)-anthracenone* ***6***

**6** was prepared from **1** (1.50 g, 6.63 mmol) in *N*-methylformanilide (10 mL) as described for **5**. Yield 11%; mp 214–216 °C; FTIR 3429, 1632 $cm^{-1}$; $^1$H-NMR ($CDCl_3$) δ 12.85 (s, 2H), 8.03 (s, 1H), 7.47–6.80 (m, 11H), 3.26 (s, 3H); MS *m/z* = 343 (88, M+). Anal $C_{22}H_{17}NO_3$ (C, H, N).

*General procedure for the preparation of substituted formanilides* ***7b–k***

To acetic anhydride (6.10 mL, 64.6 mmol) was added dropwise 98% formic acid (3.00 mL, 79.5 mmol) at 0 °C under $N_2$, and the mixture was heated to 50–60 °C for 2 h. Then it was cooled to 0 °C and treated with the appropriate aniline (20.0 mmol) in dry THF (30 mL). After 20 min the solvent was evaporated to

provide the corresponding formanilides: 4-fluoroformanilide **7b** [25], 4-chloroformanilide **7c** [25], 4-bromoformanilide **7d** [25], 4-nitroformanilide **7e** [11], 2-methoxyformanilide **7f** [26], 4-methoxyformanilide **7g** [26], 2,4-dimethoxyformanilide **7h** [26], and 3,4-dimethoxyformanilide **7i** [27].

*3,4-Methylenedioxyformanilide **7k***
Yield 82%; mp 91–94 °C (water); mixture of rotamers (1:1); FTIR 3234, 1690 $cm^{-1}$; $^1$H-NMR ($CDCl_3$) δ 8.50 (d, 1H, $^3J_{trans}$ = 11.4 Hz, C*H*O), 8.31 (d, 1H, $^3J_{cis}$ = 1.9 Hz, C*H*O), 7.96 (d, 1H, $^3J_{trans}$ = 11.4 Hz, N*H*), 7.27–6.53 (m, 6H, Ar), 7.24 (d, 1H, $^3J_{cis}$ = 1.9 Hz, N*H*), 5.99 (s, 2H, O$CH_2$O), 5.96 (s, 2H, O$CH_2$O). Anal $C_8H_7NO_3$ (C, H, N).

*General procedure for the preparation of 1,8-dihydroxy-10-[(N-phenylamino)methylene]-9(10H)-anthracenones **8a–k***

The appropriate formanilide (6.63 mmol) was dissolved in $SOCl_2$ (10 mL) under $N_2$ and stirred for 14 h at room temperature. Excess $SOCl_2$ was then removed in vacuo, the resulting foam was treated with $CH_2Cl_2$ (3 x 20 mL), and the solvent was evaporated. The residue was dissolved in dry DMPU (30 mL) under $N_2$, and the solution was added dropwise to a solution of **1** (1.50 g, 6.63 mmol) in pyridine (0.54 mL) and dry DMPU (15 mL). After the reaction was complete (TLC control), the mixture was poured into ice–water (250 mL), the resulting precipitate was collected, washed with ice–water (3 x 20 mL), and filtered by suction. The product was dissolved in $CH_2Cl_2$ (100 mL) and dried over $Na_2SO_4$. Then the solvent was evaporated and the product was purified by column chromatography using $CH_2Cl_2$.

*1,8-Dihydroxy-10-[(N-phenylamino)methylene]-9(10H)-anthracenone **8a***
The title compound was obtained as blood-red crystals; 33% yield; mp 200–202 °C; FTIR 3386, 1629 $cm^{-1}$; $^1$H-NMR ($CDCl_3$) δ 13.00 (s, 1H), 12.69 (s, 1H), 7.86 (s, 2H, NH exchangeable with $D_2O$), 7.64–6.77 (m, 11H); MS *m/z* = 329 (100, M+). Anal $C_{21}H_{15}NO_3$ (C, H, N).

*10-[(N-(4-Fluorophenyl)amino)methylene]-1,8-dihydroxy-9(10H)-anthracenone **8b***
The title compound was purified by column chromatography using $CH_2Cl_2$/hexane (3:1) and recrystallized from benzene to give orange-red crystals; 20% yield; mp 240–241 °C; FTIR 3384, 1627 $cm^{-1}$; $^1$H-NMR (DMSO–$d_6$) δ 12.97 (s, 2H), 10.13 (s, 1H), 8.30 (s, 1H), 7.78–6.77 (m, 10H); MS *m/z* = 347 (100, M+). Anal $C_{21}H_{14}FNO_3$ (C, H, N).

*10-[(N-(4-Chlorophenyl)amino)methylene]-1,8-dihydroxy-9(10H)-anthracenone **8c***
The title compound was purified by column chromatography using $CH_2Cl_2$/hexane (3:1) and recrystallized from benzene to give orange-red crystals; 28% yield; mp 255–258 °C; FTIR 3381, 1634 $cm^{-1}$; $^1$H-NMR (DMSO–$d_6$) δ 12.89 (s, 2H), 10.08 (s, 1H), 8.27 (s, 1H), 7.79–6.79 (m, 10H); MS *m/z* = 363 (100, M+). Anal $C_{21}H_{14}ClNO_3$ (C, H, N).

*10-[(N-(4-Bromophenyl)amino)methylene]-1,8-dihydroxy-9(10H)-anthracenone **8d***
The title compound was purified by column chromatography using $CH_2Cl_2$/hexane (3:1) and recrystallized from benzene to give orange-red crystals; 17% yield; mp 259–261 °C; FTIR 3381, 1627 $cm^{-1}$; $^1$H-NMR (DMSO–$d_6$) δ 12.88 (s, 2H), 10.05 (s, 1H), 8.26 (s, 1H), 7.78–6.79 (m, 10H); MS *m/z* = 407 (100, M+, $^{79}$Br), 407 (99, M+, $^{81}$Br). Anal $C_{21}H_{14}BrNO_3$ (C, H, N).

*1,8-Dihydroxy-10-[(N-(4-nitrophenyl)amino)methylene]-9(10H)-anthracenone **8e***
The title compound was obtained as blood-red crystals; 6% yield; mp 260–262 °C (dec); FTIR 3377, 1634 $cm^{-1}$; $^1$H-NMR (DMSO–$d_6$) δ 12.68 (s, 1H), 12.58 (s, 1H), 10.20 (s, 1H), 8.22–6.82 (m, 10H), 8.18 (s, 1H); MS *m/z* = 374 (100, M+). Anal $C_{21}H_{14}N_2O_5$ (C, H, N).

*1,8-Dihydroxy-10-[(N-(2-methoxyphenyl)amino)methylene]-9(10H)-anthracenone **8f***
The title compound was obtained as brick-red crystals; 24% yield; mp 180–182 °C; FTIR 3374, 1624 $cm^{-1}$; $^1$H-NMR ($CDCl_3$) δ 13.07 (s, 1H), 12.76 (s, 1H), 8.64 (d, 1H, *J* = 13.7 Hz), 7.91 (d, 1H, *J* = 13.7 Hz), 7.65–6.77 (m, 10H), 3.92 (s, 3H); MS *m/z* = 359 (100, M+). Anal $C_{22}H_{17}NO_4$ (C, H, N).

*1,8-Dihydroxy-10-[(N-(4-methoxyphenyl)amino)methylene]-9(10H)-anthracenone **8g***
The title compound was obtained as dark-red crystals; 16% yield; mp 195–196 °C; FTIR 3411, 1627 $cm^{-1}$; $^1$H-NMR ($CDCl_3$) δ 13.10 (s, 1H), 12.78 (s, 1H), 7.90 (d, 1H, exchangeable with $D_2O$), 7.80 (d, 1H), 7.63–6.76 (m, 10H), 3.82 (s, 3H); MS *m/z* = 359 (100, M+). Anal $C_{22}H_{17}NO_4$ (C, H, N).

*1,8-Dihydroxy-10-[(N-(2,4-dimethoxyphenyl)amino)methylene]-9(10H)-anthracenone **8h***
The title compound was purified by column chromatography using $CH_2Cl_2$/hexane (3:1) and recrystallized from benzene to give dark-red crystals; 8% yield; mp 216–218 °C; FTIR 3382, 1636 $cm^{-1}$; $^1$H-NMR ($CDCl_3$) δ 13.15 (s, 1H), 12.85 (s, 1H), 8.47 (d, 1H, *J* = 13.7 Hz), 7.88 (d, 1H, *J* = 13.7 Hz), 7.63–6.33 (m, 9H), 3.90 (s, 3H), 3.83 (s, 3H); MS *m/z* = 389 (100, M+). Anal $C_{23}H_{19}NO_5$ (C, H, N).

*1,8-Dihydroxy-10-[(N-(3,4-dimethoxyphenyl)amino)methylene]-9(10H)-anthracenone **8i***
The title compound was purified by column chromatography using $CH_2Cl_2$/hexane (3:1) and recrystallized from benzene to give dark-red crystals; 5% yield; mp 219–220 °C; FTIR 3313, 1621 $cm^{-1}$; $^1$H-NMR (DMSO–$d_6$) δ 13.09 (s, 2H), 10.17 (s, 1H), 8.36 (s, 1H), 7.76–6.74 (m, 9H), 3.82 (s, 3H), 3.76 (s, 3H); MS *m/z* = 389 (100, M+). Anal $C_{23}H_{19}NO_5$ (C, H, N).

*1,8-Dihydroxy-10-[(N-(3,4-methylenedioxyphenyl)amino)-methylene]-9(10H)-anthracenone **8k***
The title compound was recrystallized from $CH_2Cl_2$ to give dark-red crystals; 17% yield; mp 225–226 °C; FTIR 3276, 1630 $cm^{-1}$; $^1$H-NMR (DMSO–$d_6$) δ 13.04 (s, 2H), 10.12 (d, 1H, *J* = 8.8 Hz), 8.30 (d, 1H, *J* = 8.8 Hz), 7.77–6.74 (m, 9H), 6.05 (s, 2H); MS *m/z* = 373 (100, M+). Anal $C_{22}H_{15}NO_5$ (C, H, N).

*1,8-Dihydroxy-10-[(N-(4-hydroxyphenyl)amino)methylene]-9(10H)-anthracenone **8l***
To a solution of **8g** (0.49 g, 1.36 mmol) in dry $CH_2Cl_2$ (50 mL) at –78 °C under $N_2$ was added $BBr_3$ (1.35 mL, 13.6 mmol). The mixture was allowed to warm to room temperature in the course of 4 h. The solvent, excess reagent and methylchloride were removed in vacuo. The residue was dissolved in ether (100 mL), treated with a saturated solution of $Na_2CO_3$ (50 mL), and the mixture was extracted with ether (3 x 100 mL). The ether phase was dried over $MgSO_4$. Then hexane (10 mL) was added, and the solution was concentrated. The product was recrystallized from ether/hexane (20:1) to give dark crystals; 40% yield; mp 260–262 °C (dec); FTIR 3340, 1623 $cm^{-1}$; $^1$H-NMR (DMSO–$d_6$) δ 13.15 (s, 2H), 10.26 (br s, 1H), 9.44 (s, 1H), 8.36 (s, 1H), 7.74–6.71 (m, 10H); MS *m/z* = 345 (100, M+). Anal $C_{21}H_{15}NO_4$ (C, H, N).

*10-[(N-Benzylamino)methylene]-1,8-dihydroxy-9(10H)-anthracenone* ***9***

**9** was prepared from **1** (1.50 g, 6.63 mmol) and *N*-benzylformamide (0.90 g, 6.63 mmol) as described for **8a–k**, purified by column chromatography using $CH_2Cl_2$/hexane (4:1), and recrystallized from ethyl acetate/hexane (2:1) to give red crystals; 10% yield; mp 155–157 °C; FTIR 3415, 1617 $cm^{-1}$; $^1H$-NMR ($CDCl_3$) δ 13.20 (s, 1H), 12.93 (s, 1H), 7.54–6.48 (m, 13H), 4.54 (d, 2H, $J$ = 5.7 Hz); MS $m/z$ = 343 (23, $M^+$), 226 (100, $M^+$). Anal $C_{22}H_{17}NO_3$ (C, H, N).

*log P determination*

A standard reversed-phase HPLC procedure was used. MeOH/water/HOAc (77:23:0.1), adjusted to pH 5.5 with concentrated $NH_3$, was used as eluant. Calibration was performed as described [4]. log $P$ values as a measure of lipophilicity are given in table I.

*Bovine PMNL 5-LO assay*

Inhibition of 5-LO was determined using Ca-ionophore-stimulated bovine PMNL ($10^7$ cells/mL) as described [4]. Test compounds were preincubated for 15 min at 37 °C, and the concentrations of $LTB_4$ and 5-HETE released after 10 min were measured by reversed-phase HPLC analysis.

*Cell culture and determination of cell growth*

HaCaT cells [18] were cultivated and the cell proliferation assay was performed as previously described [5]. 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) x 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.

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