Antipsoriatic Anthrones with Modulated Redox Properties. 2. Novel Derivatives of Chrysarobin and Isochrysarobin—Antiproliferative Activity and 5-Lipoxygenase Inhibition[1]

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A novel series of 2- and 3-substituted 1,8-dihydroxy-9(10*H*)-anthracenones were synthesized and tested for their inhibitory activity against 5-lipoxygenase (5-LO) in bovine polymorphonuclear leukocytes and the growth of human keratinocytes. Structure–activity relationships are discussed with respect to the following redox properties of the compounds: reactivity against 2,2-diphenyl-1-picrylhydrazyl, generation of hydroxyl radicals as measured by deoxyribose degradation, and inhibition of lipid peroxidation in model membranes. Inhibition of cell proliferation seemed to be related to these properties, whereas 5-LO inhibition was not. Within a class of structural analogs the activity against 5-LO, which was markedly improved as compared to that of the antipsoriatic drug anthralin, correlated well with the overall lipophilicity. Even though a number of compounds in this series enhanced oxidative damage to nonlipid molecules such as deoxyribose, their antioxidant properties predominate in membrane lipids. Among the prooxidant compounds were also the most potent antiproliferative agents (IC_{50} values in the 10^{-7} M range).

The treatment of psoriasis includes the use of a wide variety of topical medications.[2] While therapeutics such as the keratolytic salicylic acid or the antiinflammatory corticosteroids are targeted toward a single feature of the disease, all psoriatic features are resolved by anthrones or coal tar in combination with UV irradiation. Among these topical agents with antipsoriatic activity, our interest has focused on anthralin (1), a related synthetic substitute of the natural product chrysarobin (2), which is the active component in Goa powder.[3] Chrysarobin had been used earlier in the treatment of psoriasis, but because of the great variation in the composition of the commercially available product, it had been replaced by anthralin. In spite of extensive research over 70 years, a more effective antipsoriatic anthrone than anthralin or chrysarobin has not yet been found.

Because of their outstanding therapeutic properties, interest continues unabated in the development of novel analogs. Like most forms of current therapy of psoriasis, the benefits of the drugs are limited by their undesirable irritant effects on the skin or poor acceptability because of staining of the skin and clothing.[3] These side effects and the mode of action of anthralin are thought to be mediated by active oxygen species and anthralin metabolite radicals.[4-9] Antipsoriatic anthrones exhibit three principal cellular effects: (1) interaction with DNA, (2) inhibition of various enzyme systems associated with cell proliferation and inflammation, and (3) redox reactions with the resulting alteration of mitochondrial functions and destruction of membrane lipids.[10] Oxygen radicals have been implicated in all these processes but are not necessarily dominant in these effects.[11] Thus, synthetic analogs to chrysarobin and anthralin with modulated generation of oxygen radicals may help elucidate the mechanism by which anthrones exert their therapeutic action and may offer an advantage in lower irritancy of the nonaffected skin.

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Lipoxygenase products of arachidonic acid metabolism were shown to be mediators of cellular infiltration and inflammation in psoriatic lesions.[12] The first step in the biosynthesis of 5-hydroxyeicosatetraenoic acid (5-HETE) and leukotriene B_4 (LTB_4) is the oxygenation of free arachidonic acid which is catalyzed by 5-lipoxygenase (5-LO).[13] As increased concentrations of LTB_4 and 5-HETE were measured in psoriatic skin,[14-17] it has been proposed that administration of 5-LO inhibitors may be therapeutically useful in the treatment of psoriasis. Thus, potential candidates for antipsoriatic therapy are evaluated for their ability to inhibit the production of LTB_4.[18-20] Considering that the biosynthesis of LTB_4 is a radical-based oxidation,[13] many 5-LO inhibitors can be categorized as antioxidants/free radical scavengers.[21-25] In addition, these compounds can remove oxygen free radicals, which mediate cell damage in a variety of skin disorders such as psoriasis.[26] According to this, dual-purpose compounds that inhibit both enzymatic (5-LO catalyzed) and nonenzymatic (oxygen-radical mediated) lipid peroxidation may be superior to individual enzyme inhibitors.

However, psoriasis involves both inflammatory and hyperproliferative processes.[27] Besides leukocyte infiltration, one major abnormality is the excessive growth of keratinocytes in the epidermis. Consequently, compounds that are targeted toward only one aspect of the disease are unlikely to be totally beneficial.[27] In vitro cultured cell systems are powerful tools in the identification of potential antipsoriatic agents having antiproliferative activity.[28]

It was anticipated that partially blocking the C-10 position of anthralin might provide agents with diminished oxygen-radical formation. We have applied such rationale to the synthesis of a series of 10-substituted 1,8-dihydroxy-9(10*H*)-anthracenones that are potent 5-LO inhibitors with modulated redox properties.[1] In particular, we pointed out that within this series there was no simple correlation between the redox properties of the compounds and the 5-LO inhibitory activity, suggesting a specific enzyme interaction rather than nonspecific redox inhibition. Analogs modified at other positions of the anthrone nucleus

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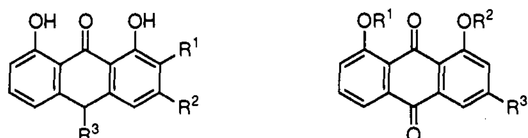
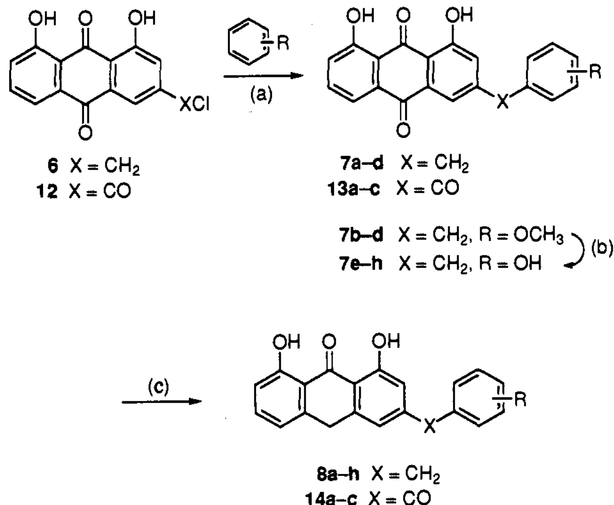
Chart 1

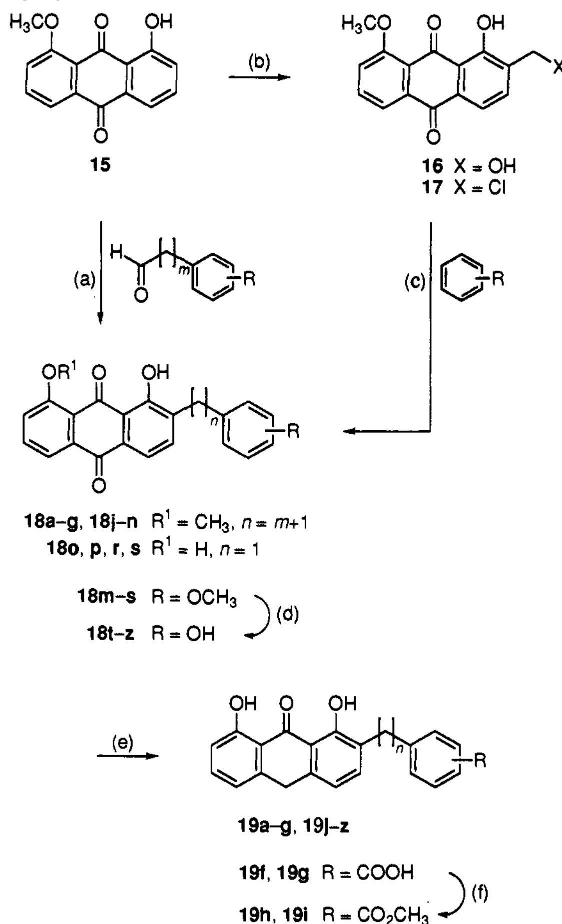


Scheme 1[a]



[a] R is defined in Tables 1 and 2. Reagents: (a) $AlCl_3$, $(CH_2)_2Cl_2$, 85 °C; (b) HBr 62%, glacial acetic acid, 118 °C; (c) $SnCl_2$, HCl, glacial acetic acid, 118 °C.

Scheme 2[a]



[a] R is defined in Table 1 and 2; m = 0–3. Reagents: (a) $Na_2S_2O_4$, NaOH, N_2, 90 °C; (b) $Na_2S_2O_4$, NaOH, HCHO, N_2, 5 °C, H_2O_2, $SOCl_2$; (c) $AlCl_3$, $(CH_2)_2Cl_2$, 85 °C; (d) HBr 62%, glacial acetic acid, 118 °C; (e) $SnCl_2$, HCl, glacial acetic acid, 118 °C; (f) MeOH, concentrated H_2SO_4.

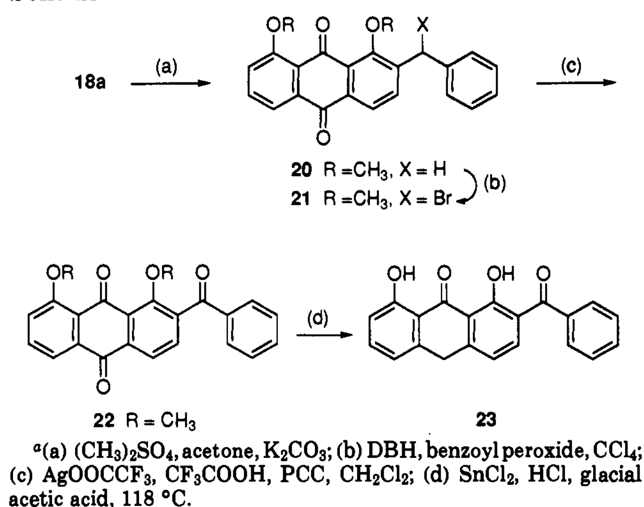
would be important for further mechanistic studies that might lead to a separation of antipsoriatic and inflammatory effects. In this paper, we describe the synthesis and biological activity of novel derivatives of chrysarobin (**2**) and isochrysarobin (**3**), 3- and 2-substituted 1,8-dihydroxy-9(10*H*)-anthracenones, respectively. Their redox properties were determined in terms of their antioxidant potential, prooxidant potential, and inhibitory effect on liposomal lipid peroxidation. Several of the new compounds are inhibitors of both enzymatic (5-LO) and nonenzymatic lipid peroxidation and exhibit potent antiproliferative activity against cell growth of human keratinocytes.

Chemistry

The presence of a hydroxymethyl functional group at C-3 of the anthracenedione nucleus of aloe emodin (**5**), readily available from oxidation of aloin (**4**),[29] suggests that this natural product might serve as a useful starting material for the synthesis of chrysarobin analogs. Treatment of aloe emodin with thionyl chloride provided the chloride **6** (Scheme 1).[30] Friedel–Crafts alkylation of appropriate benzenes with **6** afforded the corresponding 3-benzyl-1,8-dihydroxy-9,10-anthracenediones **7a–d**. The reaction of veratrole with **6** was accompanied by partial demethylation and gave an isomeric mixture of 3-hydroxy-4-methoxybenzyl- and 4-hydroxy-3-methoxybenzyl-substituted derivatives that was used in the subsequent reaction without separation. Thus, **7h** was prepared from this mixture by ether cleavage with hydrobromic acid. Analogously, the phenolic analogs **7e–g** were prepared from the methyl ethers **7b–d**. Reduction of the anthracenediones **7a–h** with stannous chloride in acetic acid/hydrochloric acid gave the desired 3-(phenylmethyl)-1,8-dihydroxy-9(10*H*)-anthracenones **8a–h** (Scheme 1). 3-Benzoyl-1,8-dihydroxy-9(10*H*)-anthracenones **14a–c** were synthesized by Friedel–Crafts acylation of an appropriately substituted benzene with rhein chloride (**12**) and selective reduction of the corresponding anthracenediones **13a–c** (Scheme 1). Rhein chloride[31] was prepared by acetylation of aloe emodin (**5**)[29] and chromic acid oxidation of the resulting triacetylaloe emodin (**9**) to diacetylrhein (**10**) followed by base-catalyzed hydrolysis to rhein (**11**)[32] and treatment with thionyl chloride (Chart 1).

Compounds possessing 2-substituents corresponding to the chrysarobin series were desired to define structure–activity relationships for this position of the molecule. Accordingly, the Marschalk reaction[33,34] was used for attaching carbon substituents to the 2-position of anthracenediones as outlined in Scheme 2. 8-Hydroxy-1-methoxy-9,10-anthracenedione (**15**) was prepared in equally good yields either by the method of Krohn[35] or from 1,8-dimethoxyanthracenedione by partial deprotection with hydrobromic acid.[36,37] The direct alkylation of **15** proceeded cleanly with benzaldehyde (m = 0) and benzaldehydes with substituents deactivating the benzenoid ring to provide the desired 2-benzyl-substituted compounds

Scheme 3[a]



[a](a) $(CH_3)_2SO_4$, acetone, K_2CO_3; (b) DBH, benzoyl peroxide, CCl_4; (c) $AgOOCCF_3$, CF_3COOH, PCC, CH_2Cl_2; (d) $SnCl_2$, HCl, glacial acetic acid, 118 °C.

18a–g. Variation in the length of the connecting chain was accomplished using the same procedure. Treatment of **15** with higher homologs of benzaldehyde (m = 1–3, Scheme 2) bearing various substituents gave the desired 2-ω-phenylalkyl-substituted analogs **18j–n**. Attempts to alkylate **15** with benzaldehydes bearing electron-donating groups failed. Thus, derivatives **18o–s** were prepared as follows. According to the route described by Krohn,[35] hydroxymethylation of **15** and reoxidation of the reaction mixture afforded the alcohol **16** which was converted into the chloride **17** (Scheme 2) by thionyl chloride. Friedel–Crafts alkylation of an appropriately substituted benzene with **17** gave the anthracenediones **18o–s**. Under these conditions, the 8-methoxy group of **17** was cleaved whereas ether groups of the aromatic ring terminating the side chain remained unchanged. However, alkylation of veratrole with **17** proceeded under partial demethylation and afforded an isomeric mixture, as already observed in the 3-substituted series. Therefore, this mixture was methylated with dimethyl sulfate to give **18q**. The phenolic derivatives **18t–z** were obtained by deprotection of the corresponding methyl ethers **18m–s** with hydrobromic acid. Final reduction of the anthracenediones as described above proceeded with concomitant ether cleavage of the 8-methoxy group of compounds **18a–g,j–n,q** and cleanly produced the corresponding anthracenones **19a–g,j–z** (Scheme 2). **19h,i** were obtained by esterification of the acids **19f,g**, respectively.

Scheme 3 shows the synthesis of the 2-benzoyl analog **23**. In an analogous manner to a literature method,[38] protection of **18a**[37] as the dimethyl ether **20**, benzylic bromination with *N,N*-dibromo-5,5-dimethylhydantoin (DBH), and treatment of **21** with silver trifluoroacetate followed by oxidation with pyridinium chlorochromate led to the anthracenedione **22**, which was then reduced with concomitant ether cleavage to the desired anthracenone **23**.

Biological Evaluation and Discussion

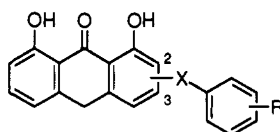
Inhibition of 5-Lipoxygenase. Inhibition of arachidonic acid 5-lipoxygenation by anthralin itself (7–74 μM, depending on cell density) has been previously reported,[39] whereas no effect against cyclooxygenase was observed.[40] In isolated bovine polymorphonuclear leukocytes (PMNL), it inhibited the production of LTB_4 and 5-HETE with an IC_{50} of 37 μM.[1] As shown in Table 1, we found that many compounds of the new series are far more potent than anthralin. The presence of phenolic groups at the terminal aromatic ring of the side chain of the anthrone strongly increases activity (e.g., **8g** and **19u,y**). This may not necessarily be related to the increased prooxidant effects of these inhibitors. For example, the carboxylic acid **19g** is devoid of hydroxyl-radical-generating properties and shows better activity than the hydroxyl-radical generator **19x**. The natural substrate arachidonic acid is metabolized as free carboxylate.[20] This is in line with the observation that the free carboxylic acids **19f,g** show greater efficacy as inhibitors than the corresponding esters **19h,i**, respectively. Furthermore, the overall lipophilicity of the esters is increased by 1 order of magnitude as compared to that of the carboxylic acids (Table 3). Therefore, 5-LO inhibitory activity appears to be due to the hydrophilic character of the molecule rather than its redox properties. Compounds too lipophilic, with log P values in general greater than 6, did not exhibit improved potency with respect to anthralin, or they simply showed limited solubility in the test system.

As shown in Table 3, we determined the log P values of the compounds by a standard HPLC procedure. These values ranged from 3.33 to 6.81. From examination of 2-benzyl-substituted analogs, it became clear that 5-LO inhibitory action increased by decreasing the overall lipophilicity of the molecule. Figure 1 shows that plots of the log IC_{50} versus log P reveal a linear dependence. Nearly parallel lines were obtained for 2-benzyl- and 2-(3-phenylpropyl)-substituted anthracenones. This relationship is in agreement with previous work[23] but in contrast to what we recently reported for a series of 10-substituted anthrones.[1]

In the 2-substituted series, the nature of the chain linking the anthrone nucleus and the phenyl ring terminus had an influence on 5-LO inhibitory action of the compounds. The 3-phenylpropyl chain was found to be superior to both the 2-phenylethyl and 4-phenylbutyl chains. Although compound **19k** is highly lipophilic with a log P of 6.44, there is still improved activity with respect to that of anthralin. Substitution at the terminal aromatic ring by hydrophilic hydroxyl groups provides a potent 5-LO inhibitor (**19u**). Replacement of the methylene group of **19a** with a keto function (**23**) enhances 5-LO inhibitory action, while substitution at the 3-position by a benzoyl group (**14a**) leaves the potency unchanged. However, hydroxyl-radical formation by **23** was not decreased as compared to that by anthralin.

Antioxidant and Prooxidant Determination. Because conversion of arachidonic acid into LTB_4 is a radical-based oxidation,[13] the interaction of potential 5-LO inhibitors with free radicals is of interest. For this purpose, we used the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH).[41] Table 1 shows that compounds with free phenolic groups on the side chain (e.g., **8g,h** and **19u,x,y**) were highly reactive against DPPH, reflecting the reducing capability of the phenolic hydroxyl. Compared to anthralin, introduction of a benzyl group in the 2- or 3-position of the anthrone nucleus (**19a** and **8a**) decreased antioxidant activity, whereas benzoyl substitution at the 3-position increased antioxidant activity. However, when the benzoyl group is moved from the 3-position (**14a**) to the 2-position (**23**) of the anthrone, the reducing capability against DPPH is dramatically increased. In this case, the resulting C-10 radical is

Table 1. Antiproliferative Activity against HaCaT Cells, 5-LO Inhibition in Bovine PMNL, and Redox Properties of 2- and 3-Substituted 1,8-Dihydroxy-9(10*H*)-anthracenones



cpd	X	R	5-LO IC_{50} (μM)[a]	k_{DPPH} (M^{-1} s^{-1})[b]	DD (μmol of MDA/mmol)[e]	LPO IC_{50} (μM)[g]	AA IC_{50} (μM)[h]
8a	3-CH_2	H	>30	18.1 ± 1.4	<0.25[f]	64	ND
8b	3-CH_2	4-OCH_3	>30	11.2 ± 2.0	<0.15	33	0.3
8c	3-CH_2	2,4-$(OCH_3)_2$	>30	10.3 ± 0.9	0.32 ± 0.14[f]	>200	0.4
8d	3-CH_2	2,5-$(OCH_3)_2$	>30	14.8 ± 0.7	0.51 ± 0.15[f]	ND	0.2
8e	3-CH_2	4-OH	>30	12.6 ± 1.8	0.27 ± 0.01[f]	14	0.4
8f	3-CH_2	2,4-$(OH)_2$	7	39.3 ± 2.7	3.26 ± 0.52[f]	16	0.5
8g	3-CH_2	2,5-$(OH)_2$	1	≫100[c]	1.74 ± 0.07[f]	3	0.4
8h	3-CH_2	3,4-$(OH)_2$	2	≫100[c]	0.41 ± 0.02[f]	4	0.5
14a	3-CO	H	>30	>100[c]	1.97 ± 0.20[f]	40	ND
14b	3-CO	4-CH_3	>30	>100[c]	1.01 ± 0.16[f]	40	ND
14c	3-CO	4-OCH_3	>30	>100[c]	0.98 ± 0.12[f]	ND	1.7
19a	2-CH_2	H	>30	11.2 ± 0.9	<0.25[f]	98	>5
19b	2-CH_2	4-CH_3	>30	8.5 ± 0.1	<0.25[f]	ND	ND
19c	2-CH_2	4-CF_3	>30	3.7 ± 0.1	<0.15	ND	>5
19d	2-CH_2	3-OPh	>30	6.4 ± 0.3	<0.15	ND	ND
19e	2-CH_2	4-Cl	>30	19.3 ± 1.0	<0.15	150	ND
19f	2-CH_2	2-COOH	4	16.9 ± 0.3	1.20 ± 0.12[f]	42	>5
19g	2-CH_2	4-COOH	2	9.9 ± 0.4	<0.25	62	2.6
19h	2-CH_2	2-CO_2CH_3	>30	11.4 ± 0.6	<0.15	ND	ND
19i	2-CH_2	4-CO_2CH_3	>30	10.4 ± 0.4	<0.25	ND	ND
19j	2-$(CH_2)_2$	H	>30	16.1 ± 0.5	<0.15	110	ND
19k	2-$(CH_2)_3$	H	11	11.1 ± 0.2	0.56 ± 0.04[f]	67	>5
19l	2-$(CH_2)_4$	H	>30	10.4 ± 0.4	<0.15[f]	112	ND
19m	2-$(CH_2)_3$	4-OCH_3	30	14.0 ± 1.3	<0.15	ND	>5
19n	2-$(CH_2)_3$	3,4,5-$(OCH_3)_3$	6	>100[c]	<0.15	ND	2.0
19o	2-CH_2	2-OCH_3	>30	12.0 ± 1.3	<0.25	ND	ND
19p	2-CH_2	4-OCH_3	>30	11.3 ± 1.2	<0.25[f]	ND	ND
19q	2-CH_2	3,4-$(OCH_3)_2$	>30	>100[c]	<0.15	ND	ND
19r	2-CH_2	2,5-$(OCH_3)_2$	>30	>100[c]	<0.15	>200	ND
19s	2-CH_2	2,4-$(OCH_3)_2$	>30	>100[c]	<0.15	ND	>5
19t	2-$(CH_2)_3$	4-OH	2	>100[c]	<0.15	50	0.4
19u	2-$(CH_2)_3$	3,4,5-$(OH)_3$	0.6	≫100[c]	2.97 ± 0.32[f]	5	0.5
19v	2-CH_2	2-OH	5	>100[c]	0.35 ± 0.04[f]	25	0.7
19w	2-CH_2	4-OH	7	>100[c]	0.38 ± 0.03[f]	43	0.8
19x	2-CH_2	3,4-$(OH)_2$	4	≫100[c]	1.82 ± 0.14[f]	4	0.4
19y	2-CH_2	2,5-$(OH)_2$	0.9	≫100[c]	1.46 ± 0.06[f]	7	0.7
19z	2-CH_2	2,4-$(OH)_2$	5	>100[c]	0.34 ± 0.04[f]	16	1.5
23	2-CO	H	18	≫100[c]	2.68 ± 0.24[f]	43	1.6
anthralin			37	24.2 ± 4.2	2.89 ± 0.14[f]	79	0.2–0.5
lonapalene			0.5	*d*	<0.15	>50	3.2

[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; $P < 0.01$. [b] Reducing activity against 2,2-diphenyl-1-picrylhydrazyl with an equimolar amount of test compound. [c] Highly reactive (approximate values). [d] Not reactive. [e] Deoxyribose degradation as a measure of hydroxyl-radical formation. Indicated values are μmol of malondialdehyde/mmol of deoxyribose released by 75 μM test compound (controls < 0.1). [f] Values are significantly different with respect to control; $P < 0.01$. [g] Inhibition of AAPH-induced lipid peroxidation in bovine brain phospholipid liposomes; $N = 3$ or more. Nordihydroguaiaretic acid (NDGA) was used as the standard ($IC_{50} = 2$ μM). [h] Antiproliferative activity against HaCaT cells. Inhibition of cell growth was significantly different with respect to that of the control; $N = 3$; $P < 0.01$. ND = not determined.

conjugated not only with the 9-keto group of the anthrone but, in addition, with the carbonyl group of the benzoyl group. Accordingly, a similar increase was observed for the prooxidant properties of this analog.

Prooxidant properties of the compounds were defined by the deoxyribose assay, which is a sensitive test for the production of hydroxyl radicals.[42] The release of malondialdehyde (MDA) is indicative of oxygen-radical formation. In general, prooxidant properties were substantially reduced with respect to those of anthralin. As already observed in the C-10 substituted series,[1] compounds with catechol, hydroquinone, or pyrogallol structure (**8g** and **19u,x,y**) once more increased the release of MDA (Table 1), indicating enhanced formation of hydroxyl radicals. Although these prooxidant analogs are good inhibitors of 5-LO, there is no simple correlation between the prooxidant or antioxidant properties and 5-LO inhibitory activity of the compounds. This is demonstrated by analogs **19f,g,k**, which show both reduced prooxidant and antioxidant properties but improved activity against 5-LO, in comparison with their predecessor anthralin.

Inhibition of Lipid Peroxidation. A variety of data have emphasized the significance of free radicals and end products derived from lipid peroxidation in inflammatory diseases. Thus, agents that inhibit initiation or propagation of lipid peroxidation may be helpful in preventing tissue injury.[43,44] The inhibitory effect on lipid peroxidation of some representative compounds was evaluated with bovine brain phospholipid liposomes which provide an ideal model system for lipid peroxidation studies.[45] Lipid peroxidation was stimulated with the azo initiator 2,2′-azobis(2-amidinopropane) hydrochloride (AAPH).[46] Indeed, compounds **8g,h** and **19u,x,y** were highly efficient in inhibition of lipid peroxidation. Their IC_{50} values ranked between 3 and 7 μM, in the same order of magnitude as that of the antioxidant nordihydroguaiaretic acid which

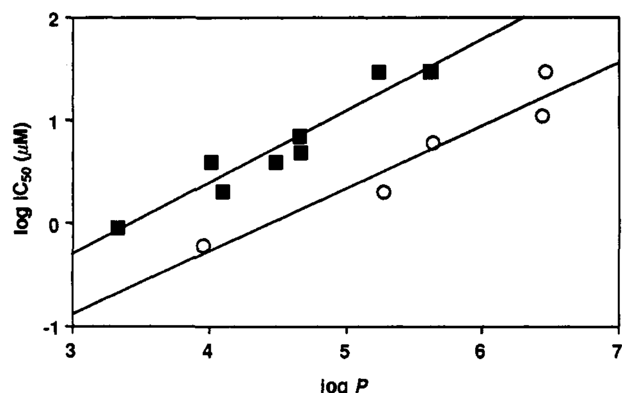


Figure 1. Linear dependence of the log of the IC_{50} (μM) for 5-LO inhibition on the log *P* of 2-substituted anthracenones: (■) 2-benzyl-1,8-dihydroxy-9(10*H*)-anthracenones, *R* = 0.93, and (○) 1,8-dihydroxy-2-(3-phenylpropyl)-9(10*H*)-anthracenones, *R* = 0.92.

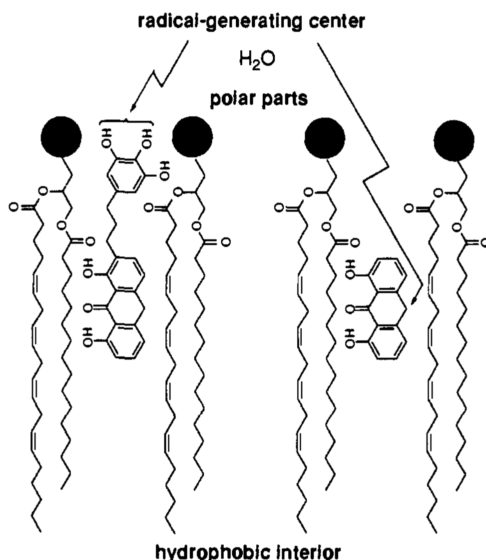


Figure 2. Model of the radical-generating center of anthralin within the hydrophobic interior of the phospholipid bilayer (one half depicted) and of **19u** at the lipid–aqueous interface. Formation of active oxygen species by anthralin enhances lipid peroxidation, whereas the pyrogallol moiety inhibits radical-induced damage to the lipid bilayer.

gave an IC_{50} of 2 μM in this test. Anthralin itself was not effective against AAPH-induced lipid peroxidation (IC_{50} = 79 μM). This is not surprising because this drug enhances lipid peroxidation both in vivo and in model membranes.[4,9]

Contrary to anthralin, none of the new phenolic analogs enhanced liposomal lipid peroxidation, despite their ability to generate hydroxyl radicals. These results can be rationalized on the basis of the orientation and position of the radical-generating site of the molecule in the phospholipid bilayer (Figure 2). As a result of the strong intramolecular hydrogen bonding between both hydroxyl functions at C-1 and C-8 and the C-9 keto group,[47] the 1,8-dihydroxy-9(10*H*)-anthracenone nucleus is even more lipophilic than the unsubstituted 9(10*H*)-anthracenone; corresponding log *P* values are 4.23 and 3.46, respectively. So, this part of the molecule is assumably fully localized in the hydrophobic region of the liposomes. According to this, the ordered arrangement of polyunsaturated fatty acids in the lipid bilayer with unsaturated centers more or less in a plane would seem to be the ideal site for an anthralin-initiated lipid peroxidation. The primary radical species generated by anthralin in lipids are formed via the C-10 position of the molecule.[7] On the other hand, radical formation by the new 2-ω-phenylalkyl-substituted derivatives is in general substantially diminished (Table 1), whereas their phenolic analogs are capable of hydroxyl-radical formation. Consequently, their radical-generating site is obviously not the C-10 position of the anthracenone nucleus that is buried within the interior of the model membranes but rather the phenolic groups at the terminating hydroquinone or the catechol groups of the C-2 side chain. These hydrophilic parts of the molecules, e.g., the pyrogallol moiety of **19u**, however, are oriented toward the water phase of the lipid bilayers. Radical generation by this molecule occurs close to the lipid–aqueous interface because it is unlikely that the polar pyrogallol moiety would enter the hydrophobic interior. Hence, the radical-generating site of **19u** does not directly come into contact with the unsaturated parts of the fatty acids within the membrane that are preferentially attacked by prooxidants. Taken together, although the phenolic analogs can enhance oxidative damage to nonlipid molecules such as deoxyribose in aqueous systems, they are not necessarily prooxidants in other systems such as lipid bilayers.

Moreover, in liposomes, their antioxidant properties predominate. Even though peroxidation of polyunsaturated fatty acid moieties leads to the formation of peroxyl radicals deep within the lipid bilayers, these hydrophilic radicals tend to be expelled from this region toward the surface of the bilayers.[48] As a consequence, one might expect an efficient inhibitor of lipid peroxidation to intercept a free radical at the lipid–aqueous interface, which is also a prime site of oxygen-radical formation.[49] This requirement is met by the assumption that the antioxidant phenolic moieties of compounds **8g,h** and **19u,x,y** are localized at this site of action and may therefore exert site-specific protection against radical species. Thus, structurally modifying the anthracenone nucleus to move away the radical-generating site from the membrane interior toward the surface of the phospholipid bilayer, hence reducing the efficacy of initiation of lipid peroxidation, may even furnish protective agents against cellular damage.

Antiproliferative Activity. A considerable body of evidence has accumulated to suggest that in vitro cultured cell systems may be useful tools in identifying new topically antipsoriatic agents.[28,50–53] As a model of epidermal hyperproliferation in psoriasis, we used HaCaT cells, a rapidly multiplying human keratinocyte line, which were described as an extremely sensitive target for the antiproliferative action of anthralin.[53] Proliferation of the keratinocytes was determined directly by counting the dispersed cells under a phase-contrast microscope after 48 h of treatment. The IC_{50} value obtained from different experiments for anthralin itself ranged between 0.2 and 0.5 μM, probably on account of the instability of this drug. Our results demonstrate that anthralin is a highly potent antiproliferative agent, and its antipsoriatic efficacy is predominantly mediated by this property rather than its 5-LO inhibitory effect. By contrast, lonapalene,[18] a 5-LO inhibitor with demonstrated topical activity against psoriasis,[54] was less potent by 1 order of magnitude, suggesting that this drug exerts its antipsoriatic effect acting primarily by 5-LO inhibition.

Although the regulation of cell proliferation is multimodal in nature, several lines of evidence suggest that

oxygen radical species may have an important role.[55] For example, evidence that anthralin-mediated damage to keratinocytes is linked to oxidative stress comes from the protective effects by concurrent treatment of the cells with superoxide dismutase and catalase.[56] Table 1 shows that the new anthrone analogs with phenolic hydroxyl groups in the attached aromatic ring exhibited consistently high activity comparable to that of anthralin but most other derivatives were in general less active or inactive. This result supports the prooxidant nature of the antiproliferative action because these compounds were all hydroxyl-radical generators. Hydroxyl-radical attack at a DNA sugar leads to sugar fragmentation, base loss, and DNA strand breakage and, finally, results in cell damage.[57,58] Further support for a prooxidant action comes from data for compounds **8c,d**, in which the phenolic hydroxyl groups are methylated. These analogs are capable of deoxyribose degradation, although diminished in comparison with that of anthralin but significantly enhanced with respect to that of the control. Compound **8b** was an exception, having unexpectedly high activity while being almost devoid of prooxidant properties.

Leukotriene B_4 is a potent stimulator of keratinocyte proliferation, which has been demonstrated in cultured human keratinocytes.[59] Within the isochrysarobin series, there seems to be a rough correlation between the inhibition of cell growth and 5-LO inhibition by the molecules. However, the antiproliferative activity did not generally correlate with the ability of the compounds to inhibit leukotriene biosynthesis. For example, the chrysarobin derivatives **8b–e**, while practically inactive as 5-LO inhibitors, were potent antiproliferative agents.

Summary and Conclusions

The development of novel antipsoriatic drugs is hindered by a lack of disease pathogenesis and no appropriate animal model. However, the activity of drugs potentially useful as antipsoriatic agents may be evaluated by their antiproliferative action in cell cultures, which may be critical in the management of the proliferative nature of psoriasis. In addition, inhibition of the generation of 5-LO products, particularly LTB_4, may be useful to resolve the inflammatory aspects of the disease. Thus, drugs having antiproliferative activity with the combined inhibitory action against both enzymatic (5-LO) and nonenzymatic lipid peroxidation could have a greater impact upon the therapeutic need than either class of drug alone. According to our study, the antipsoriatic drug anthralin is a strong inhibitor of keratinocyte growth but only a moderate 5-LO inhibitor. Contrary, lonapalene which has shown clinical efficacy in the treatment of psoriasis is a potent 5-LO inhibitor but is less efficient against keratinocyte proliferation.

Within the series of analogs substituted at the 2-position, log *P* values, as a measure of overall lipophilicity, play an important role in the activity against 5-LO. Activity is severely affected by introduction of hydrophilic groups (i.e., OH and COOH). Some of our new analogs of chrysarobin and isochrysarobin are much better inhibitors of 5-LO than anthralin, and in addition to this, they are equipotent at inhibiting growth of human keratinocytes. Moreover, while formation of anthralin radical with concomitant production of oxygen radicals results in stimulation of lipid peroxidation,[9] radical formation by compounds **8h** and **19y** is substantially reduced, and lipid peroxidation is even strongly inhibited. Although the most effective 5-LO inhibitor in this series (**19u**) is a hydroxyl-radical generator, this property does not lead to enhanced lipid peroxidation. On the contrary, good inhibitory effects were observed.

In conclusion, we have found that by altering the structure of anthralin, the in vitro profile can be markedly altered from an only moderate 5-LO inhibitor to a more potent inhibitor of this enzyme, which may reflect improved activity against the inflammatory component of psoriasis while the antiproliferative activity is retained. This structural change is also accompanied by partially diminished oxygen-radical formation and reversal of the enhancement of lipid peroxidation to potent inhibition of this process. This promises less skin-irritating properties and suggests an additional protective action against tissue injury. Thus, these compounds compared favorably in biological tests with the known antipsoriatic agent anthralin as an alternative method for treating psoriasis. Moreover, apart from their potential as antipsoriatic anthrones with reduced irritancy, these novel analogs are useful tools for mechanistic studies, and their properties suggest that structural modifications of the anthrones should continue to be explored.

Experimental Section

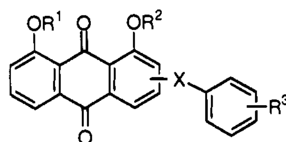
Melting points were determined with a Büchi 510 melting point apparatus and are uncorrected. Chromatography refers to column chromatography on silica gel (E. Merck, 70–230 mesh); eluants are given in Tables 2 and 3. ^{1}H NMR spectra were recorded with a Varian EM 390 (90 MHz) or Bruker Spectrospin WM 250 spectrometer (250 MHz), using tetramethylsilane as an internal standard. Fourier-transform IR spectra (KBr) were recorded on a Nicolet 510M FTIR spectrometer. UV spectra were recorded on a Kontron 810 spectrometer. Mass spectra (EI, unless otherwise stated) were obtained on a Varian MAT CH5 spectrometer (70 eV). 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).

General Procedure for Friedel–Crafts Reactions. 1,8-Dihydroxy-3-[(2,5-dimethoxyphenyl)methyl]-9,10-anthracenedione (7d). To a suspension of aloe emodin chloride[28] (**6**; 0.58 g, 2 mmol) and anhydrous $AlCl_3$ (1.1 g, 8 mmol) in 1,2-dichloroethane (20 mL) was added 1,4-dimethoxybenzene (0.41 g, 3 mmol). The reaction mixture was refluxed for 2 h (TLC control), cooled, and then poured into ice–HCl (100 mL) with stirring. The product was extracted with CH_2Cl_2 (3 × 30 mL), and the organic phase was washed with water and dried over Na_2SO_4. The solvent was evaporated, and the residue was purified by chromatography using CH_2Cl_2 to give an orange powder (Table 2): ^{1}H NMR ($CDCl_3$) δ 12.10 (s, 1H), 11.97 (s, 1H), 7.87–7.50 (m, 3H), 7.37–7.10 (m, 2H), 6.83–6.67 (m, 3H), 4.00 (s, 2H), 3.73 (s, 6H); FTIR 1675 (CO), 1629 cm^{-1} (CO···OH). Anal. ($C_{23}H_{18}O_6$) C, H.

Analogously, compounds **7a–c**, **13a–c**, and **18o–s** were prepared by reaction of appropriate benzene derivatives with **6**, **12**,[31] and **17**,[34] respectively (Table 2).

General Procedure for the Cleavage of Methyl Ethers with Hydrobromic Acid. 1,8-Dihydroxy-3-[(2,5-dihydroxyphenyl)methyl]-9,10-anthracenedione (7g). To a solution of **7d** (0.18 g, 0.50 mmol) in HOAc (10 mL) was added dropwise 62% HBr (10 mL), and the solution was refluxed for 2 h. The solution was cooled, water (20 mL) was added, and the mixture was extracted with EtOAc (3 × 50 mL). The solution was washed with $NaHCO_3$ and dried over Na_2SO_4. The solvent was evaporated, and the residue was purified by chromatography to provide a red powder (Table 2): ^{1}H NMR ($CDCl_3$/DMSO-d_6) δ 12.00 (s, 1H), 11.90 (s, 1H), 8.63 (s, 1H), 8.45 (s, 1H), 7.77–7.57 (m, 3H), 7.43–7.10 (m, 2H), 6.67–6.50 (m, 3H), 3.92 (s, 2H); FTIR 1665 (CO), 1627 cm^{-1} (CO···OH); MS *m/z* 362 (100). Anal. ($C_{21}H_{14}O_6$) C, H.

Table 2. Chemical Data of 2- and 3-Substituted 1,8-Dihydroxy-9,10-anthracenediones



cpd[a]	R^1	R^2	X	R^3	mp (°C)	yield (%)	chromatography solvent (vol %)[c]	formula
7a	H	H	3-CH_2	H	143–144	30	T	$C_{21}H_{14}O_4$
7b	H	H	3-CH_2	4-OCH_3	189–190	28	PE/EA (9–1)	$C_{22}H_{16}O_5$
7c	H	H	3-CH_2	2,4-$(OCH_3)_2$	159–160	12	MC/H (4–1)	$C_{23}H_{18}O_6$
7d	H	H	3-CH_2	2,5-$(OCH_3)_2$	187–188	50	MC	$C_{23}H_{18}O_6$
7e	H	H	3-CH_2	4-OH	119–120	42	MC/H (4–1)	$C_{21}H_{14}O_5$
7f	H	H	3-CH_2	2,4-$(OH)_2$	219–220	56	MC/EA (4–1)	$C_{21}H_{14}O_6$
7g	H	H	3-CH_2	2,5-$(OH)_2$	220–221	66	MC/EA (4–1)	$C_{21}H_{14}O_6$
7h	H	H	3-CH_2	3,4-$(OH)_2$	215–216	15	MC/EA (7–3)	$C_{21}H_{14}O_6$
13a	H	H	3-CO	H	166–168	14	MC	$C_{21}H_{12}O_5$
13b	H	H	3-CO	4-CH_3	168–170	15	MC	$C_{22}H_{14}O_5$
13c	H	H	3-CO	4-OCH_3	116–118	53	MC	$C_{22}H_{14}O_6$
18a[37]	CH_3	H	2-CH_2	H	187–188	24	MC	$C_{22}H_{16}O_4$
18b	CH_3	H	2-CH_2	4-CH_3	170–171	23	MC	$C_{23}H_{18}O_4$
18c	CH_3	H	2-CH_2	4-CF_3	213–215	19	MC	$C_{23}H_{15}F_3O_4$
18d	CH_3	H	2-CH_2	3-OPh	158–159	15	MC	$C_{28}H_{20}O_5$
18e	CH_3	H	2-CH_2	4-Cl	191–193	15	MC	$C_{22}H_{15}ClO_4$
18f	CH_3	H	2-CH_2	2-COOH	224–226	26	EA/P (4–1)	$C_{23}H_{16}O_6$
18g	CH_3	H	2-CH_2	4-COOH	270[b]	27	EA/P (4–1)	$C_{23}H_{16}O_6$
18h	CH_3	H	2-CH_2	2-CO_2CH_3	150–151	48	MC	$C_{24}H_{18}C_6$
18i	CH_3	H	2-CH_2	4-CO_2CH_3	220–221	67	MC	$C_{24}H_{18}O_6$
18j	CH_3	H	2-$(CH_2)_2$	H	234–235	26	MC	$C_{23}H_{18}O_4$
18k	CH_3	H	2-$(CH_2)_3$	H	125–127	18	MC	$C_{24}H_{20}C_4$
18l	CH_3	H	2-$(CH_2)_4$	H	132–133	37	MC	$C_{25}H_{22}O_4$
18m	CH_3	H	2-$(CH_2)_3$	4-OCH_3	174–175	17	MC/EA (9–1)	$C_{25}H_{22}O_5$
18n	CH_3	H	2-$(CH_2)_3$	3,4,5-$(OCH_3)_3$	155–156	22	MC/EA (19–1)	$C_{27}H_{26}O_7$
18o	H	H	2-CH_2	2-OCH_3	195–196	13	MC/H (3–2)	$C_{22}H_{16}O_5$
18p	H	H	2-CH_2	4-OCH_3	169–171	23	MC/H (3–2)	$C_{22}H_{16}O_5$
18q	CH_3	CH_3	2-CH_2	3,4-$(OCH_3)_2$	148–149	88	MC/EA (9–1)	$C_{25}H_{22}O_6$
18r	H	H	2-CH_2	2,5-$(OCH_3)_2$	167–168	30	MC	$C_{23}H_{18}O_6$
18s	H	H	2-CH_2	2,4-$(OCH_3)_2$	167–169	34	MC	$C_{23}H_{18}O_6$
18t	H	H	2-$(CH_2)_3$	4-OH	181–182	53	MC/EA (9–1)	$C_{23}H_{18}O_5$
18u	H	H	2-$(CH_2)_3$	3,4,5-$(OH)_3$	226–227	45	MC/EA (4–1)	$C_{23}H_{18}O_7$
18v	H	H	2-CH_2	2-OH	258–259	48	MC	$C_{21}H_{14}O_5$
18w	H	H	2-CH_2	4-OH	217–218	61	MC	$C_{21}H_{14}O_5$
18x	H	H	2-CH_2	3,4-$(OH)_2$	219–221	35	MC/EA (4–1)	$C_{21}H_{14}O_6$
18y	H	H	2-CH_2	2,5-$(OH)_2$	219–220	53	MC/EA (4–1)	$C_{21}H_{14}O_6$
18z	H	H	2-CH_2	2,4-$(OH)_2$	254–255	27	MC/EA (4–1)	$C_{21}H_{14}O_6$
20	CH_3	CH_3	2-CH_2	H	138–139	85	MC	$C_{23}H_{18}O_4$
22	CH_3	CH_3	2-CO	H	200–202	21	MC	$C_{23}H_{16}O_5$

[a] All new compounds displayed ^{1}H NMR, FTIR, UV, and MS spectra consistent with the assigned structure. Elemental analyses (C, H) were within ±0.4% of calculated values except where stated otherwise. [b] Decomposition. [c] E = ether; EA = ethyl acetate; H = hexane; M = methanol; MC = methylene chloride; P = petroleum ether (40–60); T = toluene.

Compounds **7e,f** and **18t–z** were prepared from the corresponding methyl ethers (**7b,c** and **18m–s**, respectively) as described above (Table 2). **7h** was prepared from the isomeric mixture obtained by reaction of **6** with veratrole.

General Procedure for the Marschalk Reaction of 1-Hydroxy-8-methoxy-9,10-anthracenedione (15) with Various Benzaldehydes. 1-Hydroxy-8-methoxy-2-(3-phenylpropyl)-9,10-anthracenedione (18k). To a solution of 1.5% NaOH in water (120 mL) were added **15**[30] (2 g, 7.9 mmol) and a solution of $Na_2S_2O_4$ (2.4 g, 11.7 mmol) in water (15 mL) under nitrogen. The solution was heated to 50 °C for 15 min. 3-Phenylpropionaldehyde (2.12 g, 15.8 mmol) was added, and the temperature was raised to 90 °C. After 3 h, the reaction mixture was cooled, aerated for 15 min, and then acidified with 22% HCl. The precipitate thus obtained was extracted with CH_2Cl_2 (3 × 200 mL); the organic phase was washed with water and dried over Na_2SO_4. The solvent was evaporated, and the residue was purified by chromatography to provide an orange-red powder (Table 2): ^{1}H NMR ($CDCl_3$) δ 13.35 (s, 1H), 8.00–7.00 (m, 10H), 4.08 (s, 3H), 3.00–2.50 (m, 4H), 2.19–1.88 (m, 2H); FTIR 1670 (CO), 1634 cm^{-1} (CO···OH). Anal. ($C_{24}H_{20}O_4$) C, H.

According to this method, analogs **18a–g,j–n** were prepared (Table 2). Starting materials for **18l–n** were 4-phenylbutyraldehyde,[60] 3-(4-methoxyphenyl)propionaldehyde,[61] and 3-(3,4,5-trimethoxyphenyl)propionaldehyde, respectively.

3-(3,4,5-Trimethoxyphenyl)propionaldehyde. Via the method of Corey,[62] a solution of 3-(3,4,5-trimethoxyphenyl)-propanol[63] (18 g, 80 mmol) in dry CH_2Cl_2 (16 mL) was added to pyridinium chlorochromate (26 g, 120 mmol) in CH_2Cl_2 (160 mL), and the mixture was stirred for 90 min. Dry ether (160 mL) was then added and the supernatant decanted from the precipitate. The residue was washed thoroughly with dry ether (3 × 50 mL), and the organic phase was filtered through silica gel. Evaporation of the solvent gave a pale yellow oil (16.2 g, 90%): bp 173–176 °C (666.6 Pa); ^{1}H NMR ($CDCl_3$) δ 9.85 (s, 1H), 6.40 (s, 2H), 3.85 (s, 9H), 2.85–2.72 (m, 4H); FTIR 1720 cm^{-1} (CO).

General Procedure for the Reduction of 1,8-Dihydroxy-9,10-anthracenediones to the Corresponding 1,8-Dihydroxy-9(10*H*)-anthracenones. 1,8-Dihydroxy-3-[(2,5-dihydroxyphenyl)methyl]-9(10*H*)-anthracenone (8g). According to the method of Auterhoff and Scherff,[64] to a solution of compound **7g** (0.25 g, 0.70 mmol) in glacial HOAc (20 mL) heated to reflux was added, dropwise over 3 h, a solution of 40% $SnCl_2$ in 37% HCl (5.2 mL). The solution was then cooled, and the resulting crystals were collected by filtration. Purification by chromatography provided a yellow powder (Table 3): ^{1}H NMR (DMSO-d_6) δ 12.27 (s, 1H), 12.13 (s, 1H), 8.37 (s, 1H), 8.30 (s, 1H), 7.63–7.37 (m, 1H), 7.03–6.47 (m, 7H), 4.33 (s, 2H), 3.87 (s, 2H); FTIR 1623 cm^{-1} (CO···OH); MS m/z 348 (100). Anal. ($C_{21}H_{16}O_5$) C, H.

Analogs **8a–f,h**, **14a–c**, **19a–g,j–z**, and **23** were prepared from **7a–f,h**, **13a–c**, **18a–g,j–z**, and **22**, respectively, according to the method described above (Table 3).

Table 3. Chemical Data of 2- and 3-Substituted 1,8-Dihydroxy-9(10*H*)-anthracenones

cpd[a]	log *P*	mp (°C)	yield (%)	chromatography solvent (vol %)[c]	formula
8a	5.29	147–148	80	T	$C_{21}H_{16}O_3$
8b	5.30	173–174	52	T	$C_{22}H_{18}O_4{\cdot}H_2O$
8c	5.34	173–174	52	MC	$C_{23}H_{20}O_5$
8d	5.17	168–169	65	MC	$C_{23}H_{20}O_5{\cdot}0.25H_2O$
8e	4.52	170–171	46	MC	$C_{21}H_{16}O_4$
8f	3.67	141–142	37	MC/EA (7–3)	$C_{21}H_{16}O_5{\cdot}0.5H_2O$
8g	3.20	215–216	46	MC/EA (4–1)	$C_{21}H_{16}O_5{\cdot}0.25H_2O$
8h	3.88	220[b]	59	MC/EA (9–1)	$C_{21}H_{16}O_5$
14a	4.83	183–184	52	MC	$C_{21}H_{14}O_4$
14b	5.14	188[b]	81	T	$C_{22}H_{16}O_4{\cdot}H_2O$
14c	4.83	168–169	76	T	$C_{22}H_{16}O_5{\cdot}H_2O$
19a	5.61	163–164	71	MC/H (7–2)	$C_{21}H_{16}O_3$
19b	6.04	163–164	75	MC/H (7–2)	$C_{22}H_{16}O_3$
19c	5.92	185–187	71	MC/H (7–2)	$C_{22}H_{15}F_3O_3$
19d	6.35	160–161	67	MC/H (7–2)	$C_{27}H_{20}O_4$
19e	5.98	174–175	63	MC/H (7–2)	$C_{21}H_{15}ClO_3$
19f	4.48	226–227	62	MC/E (7–3)	$C_{22}H_{16}O_5$
19g	4.10	245[b]	69	MC/E (7–3)	$C_{22}H_{16}O_5$
19h	5.24	198–199	43	MC/H (4–1)	$C_{23}H_{18}O_5$
19i	5.34	181–182	30	MC/H (4–1)	$C_{23}H_{18}O_5$
19j	6.23	129–130	70	MC	$C_{22}H_{18}O_3$
19k	6.44	99–101	75	MC	$C_{23}H_{20}O_3$
19l	6.81	125–126	59	MC	$C_{24}H_{22}O_3$
19m	6.46	114–115	53	MC	$C_{24}H_{22}O_4$
19n	5.64	148–149	86	MC/EA (19–1)	$C_{26}H_{26}O_8$
19o	5.62	182–183	58	MC/H (7–2)	$C_{22}H_{18}O_4$
19p	5.62	165–166	74	MC/H (7–2)	$C_{22}H_{18}O_4$
19q	5.79	135–137	28	MC	$C_{23}H_{20}O_5$
19r	5.72	164–165	30	MC	$C_{23}H_{20}O_5$
19s	5.04	135–137	25	MC	$C_{23}H_{20}O_5$
19t	5.27	161–162	50	MC/EA (9–1)	$C_{23}H_{20}O_4$
19u	3.96	195–196	62	E/M (9–1)	$C_{23}H_{20}O_6$
19v	4.67	188–190	72	MC/E (9–1)	$C_{21}H_{16}O_4$
19w	4.66	180–181	78	MC/E (9–1)	$C_{21}H_{16}O_4$
19x	4.01	188–189	47	MC/EA (3–1)	$C_{21}H_{16}O_5$
19y	3.33	178–179	47	MC/EA (4–1)	$C_{21}H_{16}O_5$
19z	3.68	208–210	36	MC/E (8–1)	$C_{21}H_{16}O_5$
23	4.47	185–186	62	MC	$C_{21}H_{14}O_4$

[a–c] See footnotes of Table 2.

1,8-Dihydroxy-2-[[4-(methoxycarbonyl)phenyl]methyl]-9(10*H*)-anthracenone (19i). A solution of compound **19g** (1.0 g, 2.77 mmol) in absolute methanol (300 mL) and 96% H_2SO_4 (1 mL) was refluxed for 24 h. The reaction was then cooled to −20 °C, and the resulting crystals were filtered by suction. Purification by chromatography gave yellow crystals (Table 3): ^{1}H NMR ($CDCl_3$) δ 12.72 (s, 1H), 12.35 (s, 1H), 8.02–6.78 (m, 9H), 4.38 (s, 2H), 4.02 (s, 2H), 3.90 (s, 3H); FTIR 1726 (CO_2CH_3), 1618 cm^{-1} (CO···OH); MS *m/z* 374 (100). Anal. ($C_{23}H_{18}O_5$) C, H.

Analogously, **19h** was prepared from **19f** (Table 3).

1,8-Dimethoxy-2-(phenylmethyl)-9,10-anthracenedione (20). To a suspension of **18a**[37] (1.0 g, 2.90 mmol) and K_2CO_3 (1.2 g, 0.87 mmol) in dry acetone (35 mL) heated to reflux was added, dropwise over 1 h, dimethyl sulfate (2.65 g, 21.0 mmol). After 30 min, another portion of K_2CO_3 (1.2 g, 0.87 mmol) was added, and the reflux was continued for 3 h. The reaction mixture was then poured into a mixture of ice (35 g) and 37% HCl (4 mL). The resulting precipitate was filtered by suction and dried over phosphorus pentoxide. Purification by chromatography provided yellow crystals (Table 2): ^{1}H NMR ($CDCl_3$) δ 7.99–7.24 (m, 10H), 4.15 (s, 2H), 4.03 (s, 3H), 3.90 (s, 3H); FTIR 1674 cm^{-1} (CO). Anal. ($C_{23}H_{18}O_5$) C, H.

2-Benzoyl-1,8-dimethoxy-9,10-anthracenedione (22). A suspension of **20** (1.0 g, 2.79 mmol) and 1,3-dibromo-5,5-dimethylhydantoin (0.71 g, 2.46 mmol) in dry CCl_4 (50 mL) was refluxed for 3 h. The reaction was then cooled and the solvent evaporated. The crude product (**21**) was used directly in the next step without further purification.

Bromide **21** (1.0 g, 2.28 mmol), silver trifluoroacetate (0.7 g, 3.15 mmol), and CF_3COOH (5 mL) were stirred for 30 min; then, the solvent was evaporated. Dry $CHCl_3$ was added, and the insoluble salts were filtered off. The solvent was evaporated and the residue dissolved in dry CH_2Cl_2 (5 mL). Pyridinium chlorochromate (0.7 g, 3.25 mmol) was added, and the mixture was stirred for 90 min. Ether (20 mL) was then added, and the solution was filtered through silica gel. The solvent was evaporated, and the residue was purified by chromatography to provide red crystals (Table 2): ^{1}H NMR ($CDCl_3$) δ 8.18–7.28 (m, 10H), 4.03 (s, 3H), 3.85 (s, 3H); FTIR 1672 cm^{-1} (CO); MS *m/z* 372 (53). Anal. ($C_{23}H_{16}O_5$) C, H.

log *P* Determination.[65] 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.[1]

Determination of the Reducing Activity against 2,2-Diphenyl-1-picrylhydrazyl.[9] To 1 mL of the test compound solution (10^{-4} M) was added 1 mL of DPPH solution (10^{-4} M), each in acetone/PBS (1–1 v/v), and the reduction of DPPH was followed spectrophotometrically at 516 nm. Plots of the reciprocal of DPPH concentrations against time gave straight lines, and the second-order rate constants were obtained from the slopes and are expressed as mean values (N = 3–6).

Degradation of 2-Deoxy-D-ribose. The deoxyribose assay was conducted as described.[1] 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 $FeCl_3{\cdot}6H_2O$ (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.

Assay of Lipid Peroxidation. Inhibition of lipid peroxidation in bovine brain phospholipid liposomes was performed essentially as described.[1] The following reagents were added at the final concentrations stated: 0.3 mL of KH_2PO_4–KOH buffer, pH 7.4 (30 mM), 0.39 mL of H_2O (double distilled), 0.2 mL of liposomes (1 mg/mL), 0.1 mL of AAPH (10 mM), and 0.01 mL of anthracenone derivative (variable concentrations). After incubation for 1 h at 37 °C, TBA-reactive material was measured at 532 nm.

Bovine PMNL 5-Lipoxygenase Assay. Inhibition of 5-LO was determined using Ca-ionophore-stimulated bovine PMNL (10^7 cells/mL) as previously described.[1] 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 were kindly provided by Dr. R. Hein, Dermatologische Klinik, Universität Regensburg. The cells were grown in Dulbecco's modified Eagle's medium (DMEM, No. 041-11965A, Gibco) supplemented with 10% fetal calf serum, penicillin (100 units/mL), and streptomycin (100 μg/mL) in a humidified incubator containing 8% CO_2 at 37 °C. Cells (2.5×10^4/1.1 mL suspension per well) were seeded on 24-well multidishes and grown in DMEM. After 24-h growth, the medium was replaced, and the test compounds (0.1–5 μM) were added from stock solutions. These were prepared in DMSO and then diluted with DMEM; the final concentration of DMSO was 0.2% in the culture medium. Controls were performed with DMSO or medium alone. Forty-eight hours after addition of the test compounds to the culture, the medium was removed and each well was rinsed with phosphate-buffered saline (PBS, 100 μL). The cells were then incubated with sterile 0.5% trypsin and 0.2% EDTA in PBS for 20 min at 37 °C. The detached cells from each well were suspended in DMEM and dispersed into single cells by gentle pipetting through an Eppendorf pipette, and cell growth was determined directly by counting the keratinocytes. The cells were enumerated in Neubauer counting chambers 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.

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References

(1) Müller, K.; Gürster, D.; Piwek, S.; Wiegrebe, W. Antipsoriatic anthrones with modulated redox properties. 1. Novel 10-substituted 1,8-dihydroxy-9(10*H*)-anthracenones as inhibitors of 5-lipoxygenase. *J. Med. Chem.* **1993**, *36*, 4099–4107.

(2) Farber, E. M.; Nall, L. Psoriasis. A review of recent advances in treatment. *Drugs* 1984, *28*, 324–346.

(3) Ashton, R. E.; Andre, P.; Lowe, N. J.; Whitefield, M. Anthralin: historical and current perspectives. *J. Am. Acad. Dermatol.* 1983, *9*, 173–192.

(4) Müller, K.; Wiegrebe, W.; Younes, M. Formation of active oxygen species by dithranol, III. Dithranol, active oxygen species and lipid peroxidation in vivo. *Arch. Pharm. (Weinheim, Ger.)* 1987, *320*, 59–66.

(5) Müller, K.; Kappus, H. Hydroxyl radical formation by dithranol. *Biochem. Pharmacol.* 1988, *37*, 4277–4280.

(6) Fuchs, J.; Packer, L. Investigations on anthralin free radicals in model systems and in skin of hairless mice. *J. Invest. Dermatol.* 1989, *92*, 677–682.

(7) Lambelet, P.; Ducret, F.; Löliger, J.; Maignan, J.; Reichert, U.; Shroot, B. The relevance of secondary radicals in the mode of action of anthralin. *Free Radical Biol. Med.* 1990, *9*, 183–190.

(8) Müller, K.; Kanner, R. C.; Foote, C. S. Kinetic studies on anthralin photooxidation. *Photochem. Photobiol.* **1990**, *52*, 445–450.

(9) Müller, K.; Gürster, D. Hydroxyl radical damage to DNA sugar and model membranes induced by anthralin (dithranol). *Biochem. Pharmacol.* **1993**, *46*, 1695–1704.

(10) Kemény, L.; Ruzicka, T.; Braun-Falco, O. Dithranol: a review of the mechanism of action in the treatment of psoriasis vulgaris. *Skin Pharmacol.* **1990**, *3*, 1–20.

(11) Hayden, P. J.; Chignell, C. F. Detection and characterization of 9-anthron-10-yl radicals formed by antipsoriatic and tumor-promoting 9-anthrones in aqueous buffers. *Chem. Res. Toxicol.* **1993**, *6*, 231–237.

(12) Ford-Hutchinson, A. W. Potential and therapeutical value of development of novel 5-lipoxygenase inhibitors. In *Lipoxygenases and Their Products*; Cooke, S. T., Wong, A., Eds.; Academic Press: London, 1991; pp 137–160.

(13) Musser, J. H.; Kreft, A. F. 5-Lipoxygenase: properties, pharmacology, and the quinolinyl(bridged)aryl class of inhibitors. *J. Med. Chem.* **1992**, *35*, 2501–2524.

(14) Brain, S. D.; Camp, R. D. R.; Cunningham, F. M.; Dowd, P. M.; Greaves, M. W.; Kobza Black, A. Leukotriene B_4-like material in scale of psoriatic skin lesions. *Br. J. Pharmacol.* **1984**, *83*, 313–317.

(15) Grabbe, J.; Czarnetzki, B. M.; Rosenbach, T.; Mardin, M. Identification of chemotactic lipoxygenase products of arachidonate metabolism in psoriatic skin. *J. Invest. Dermatol.* **1984**, *82*, 477–479.

(16) Duell, E. A.; Ellis, C. N.; Voorhees, J. J. Determination of 5,12, and 15-lipoxygenase products in keratomed biopsies of normal and psoriatic skin. *J. Invest. Dermatol.* 1988, *91*, 446–450.

(17) Fogh, K.; Herlin, T.; Kragballe, K. Eicosanoids in acute and chronic psoriatic lesions: Leukotriene B_4, but not 12-hydroxy-eicosatetraenoic acid, is present in biologically active amounts in acute guttate lesions. *J. Invest. Dermatol.* **1989**, *92*, 837–841.

(18) Jones, G. H.; Venuti, M. C.; Young, J. M.; Krishna Murthy, D. V.; Loe, B. E.; Simpson, R. A.; Berks, A. H.; Spires, D. A.; Maloney, P. J.; Kruseman, M.; Rouhafza, S.; Kappas, K. C.; Beard, C. C.; Unger, S. H.; Cheung, P. S. Topical nonsteroidal antipsoriatic agents: 1. 1,2,3,4-Tetraoxygenated naphthalene derivatives. *J. Med. Chem.* **1986**, *29*, 1504–1511.

(19) Venuti, M. C.; Loe, B. E.; Jones, G. H.; Young, J. M. Topical nonsteroidal antipsoriatic drugs. 2. 2,3-(Alkylidenedioxy)naphthalene analogues of lonapalene. *J. Med. Chem.* 1988, *31*, 2132–2136.

(20) Batt, D. G.; Maynard, G. D.; Petraitis, J. J.; Shaw, J. E.; Galbraith, W.; Harris, R. R. 2-Substituted-1-naphthols as potent 5-lipoxygenase inhibitors with topical antiinflammatory activity. *J. Med. Chem.* **1990**, *33*, 360–370.

(21) Ikuta, H.; Shirota, H.; Kobayashi, S.; Yamagishi, Y.; Yamada, K.; Yamatsu, I.; Katayama, K. Synthesis and antiinflammatory activities of 3-(3,5-di-tert-butyl-4-hydroxybenzylidene)pyrrolidin-2-ones. *J. Med. Chem.* 1987, *30*, 1995–1998.

(22) Hammond, M. L.; Kopka, I. E.; Zambias, R. A.; Caldwell, C. G.; Boger, J.; Baker, F.; Bach, T.; Luell, S.; MacIntyre, D. E. 2,3-Dihydro-5-benzofuranols as antioxidant-based inhibitors of leukotriene biosynthesis. *J. Med. Chem.* **1989**, *32*, 1006–1020.

(23) Hammond, M. L.; Zambias, R. A.; Chang, M. N.; Jensen, N. P.; McDonald, J.; Thompson, K.; Boulton, D. A.; Kopka, I. E.; Hand, K. M.; Opas, E. E.; Luell, S.; Bach, T.; Davies, P.; MacIntyre, D. E.; Bonney, R. J.; Humes, J. L. Antioxidant-based inhibitors of leukotriene biosynthesis. The discovery of 6-[1-[2-(hydroxymethyl)-phenyl]-1-propen-3-yl]-2,3-dihydro-5-benzofuranol, a potent topical antiinflammatory agent. *J. Med. Chem.* **1990**, *33*, 908–918.

(24) Lazer, E. S.; Wong, H.-C.; Possanza, G. J.; Graham, A. G.; Farina, P. R. Antiinflammatory 2,6-di-tert-butyl-4-(2-arylethenyl)phenols. *J. Med. Chem.* **1989**, *32*, 100–104.

(25) Lazer, E. S.; Wong, H.-C.; Wegner, C. D.; Graham, A. G.; Farina, P. R. Effect of structure on potency and selectivity in 2,6-disubstituted 4-(2-arylethenyl)phenol lipoxygenase inhibitors. *J. Med. Chem.* **1990**, *33*, 1892–1898.

(26) Trenam, C. W.; Dabbagh, A. J.; Morris, C. J.; Blake, D. R. Skin inflammation induced by reactive oxygen species (ROS): an in-vivo model. *Br. J. Dermatol.* **1991**, *125*, 325–329.

(27) Ziboh, V. A. Psoriasis: Hyperproliferative/inflammatory skin disorder. *Drug Dev. Res.* **1988**, *13*, 137–146.

(28) Klem, E. B. Effects of antipsoriasis drugs and metabolic inhibitors on the growth of epidermal cells in culture. *J. Invest. Dermatol.* 1978, *70*, 27–32.

(29) Cahn, R. S.; Simonsen, J. L. Experiments on the constitution of the aloins. Part III. *J. Chem. Soc.* **1932**, 2573–2582.

(30) Krohn, K.; Baltus, W. Note on the synthesis of benz[*a*]anthracenequinones. *Liebigs Ann. Chem.* **1982**, 1579–1581.

(31) Oesterle, O. A.; Haugseth, E. R. Certain derivatives of rhein. *Arch. Pharm. (Weinheim, Ger.)* **1915**, *253*, 330–334.

(32) Robinson, R.; Simonsen, J. L. Experiments on the constitution of the aloins. Part I. *J. Chem. Soc.* **1909**, *95*, 1085–1096.

(33) Marschalk, C.; Koenig, F.; Ouroussoff, N. A new method of introducing side chains into the anthraquinone nucleus. *Bull. Soc. Chim. Fr.* **1936**, *3*, 1545–1575.

(34) Krohn, K. Synthesis of anthracyclinones by electrophilic and nucleophilic addition to anthraquinones. *Tetrahedron* **1990**, *46*, 291–318.

(35) Krohn, K.; Müller, U.; Priyono, W.; Sarstedt, B.; Stoffregen, A. Intramolecular addition of carbanions to anthraquinones. *Liebigs Ann. Chem.* **1984**, 306–318.

(36) Koyama, M.; Takahashi, K.; Chou, T.-C.; Darzynkiewicz, Z.; Kapuscinski, J.; Kelly, T. R.; Watanabe, K. A. Intercalating agents with covalent bond forming capability. A novel type of potential anticancer agents. 2. Derivatives of chrysophanol and emodin. *J. Med. Chem.* **1989**, *32*, 1594–1599.

(37) Zembower, D. E.; Kam, C.-M.; Powers, J. C.; Zalkow, L. H. Novel anthraquinone inhibitors of human leukocyte elastase and cathepsin G. *J. Med. Chem.* **1992**, *35*, 1597–1605.

(38) Cava, M. P.; Ahmed, Z.; Benfaremo, N.; Murphy, R. A., Jr.; OMalley, G. J. Anthraquinone dye intermediates as precursors of aklavinone-type anthracyclinones. *Tetrahedron* **1984**, *40*, 4767–4776.

(39) Schröder, J.-M. Anthralin (1,8-dihydroxyanthrone) is a potent inhibitor of leukotriene production and LTB4-ω oxidation by human neutrophils. *J. Invest. Dermatol.* **1986**, *87*, 624–629.

(40) Bedord, C. J.; Young, J. M.; Wagner, B. M. Anthralin inhibition of mouse epidermal arachidonic acid lipoxygenase in vitro. *J. Invest. Dermatol.* **1983**, *81*, 566–571.

(41) Blois, M. S. Antioxidant determinations by the use of a stable free radical. *Nature* **1958**, *181*, 1199–1200.

(42) Halliwell, B.; Grootveld, M.; Gutteridge, J. M. C. Methods for the measurement of hydroxyl radicals in biochemical systems: deoxyribose degradation and aromatic hydroxylation. *Methods Biochem. Anal.* **1988**, *33*, 59–90.

(43) Fraisse, L.; Verlhac, J.-B.; Roche, B.; Rascle, M. C.; Rabion, A.; Seris, J. L. Long-chain-substituted uric acid and 5,6-diaminouracil derivatives as novel agents against free radical processes: synthesis and in vitro activity. *J. Med. Chem.* **1993**, *36*, 1465–1473.

(44) Yu, M. J.; McGowan, J. R.; Phebus, L. A.; Towner, R. D.; Ho, P. K.; Keith, P. T.; Luttman, C. A.; Saunders, R. D.; Ruterbories, K. J.; Lindstrom, T. D.; Wikel, J. H.; Morgan, E.; Hahn, R. A. Benzylamine antioxidants: relationship between structure, peroxyl radical scavenging, lipid peroxidation inhibition, and cytoprotection. *J. Med. Chem.* **1993**, *36*, 1262–1271.

(45) Gutteridge, J. M. C. The measurement of malondialdehyde in peroxidised ox-brain phospholipid liposomes. *Anal. Biochem.* **1977**, *82*, 76–82.

(46) Niki, E. Free radical initiators as source of water- or lipid-soluble peroxyl radicals. *Methods Enzymol.* **1990**, *186*, 100–108.

(47) Avdovich, H. W.; Neville, G. A. 1,8-dihydroxy-9-anthrone, the revised structure for anthralin, a U.S.P. reference standard. *Can. J. Spectrosc.* 1980, *25*, 110–113.

(48) Barclay, L. R. C.; Ingold, K. U. Autoxidation of biological molecules. 2. The autoxidation of a model membrane. A comparison of the autoxidation of egg lecithin phosphatidylcholine in water and in chlorobenzene. *J. Am. Chem. Soc.* **1981**, *103*, 6478–6485.

(49) Jamieson, D. Oxygen toxicity and reactive oxygen metabolites in mammals. *Free Radical Biol. Med.* **1989**, *7*, 87–108.

(50) Clark, J. M.; Hanawalt, P. C. Inhibition of DNA replication and repair by anthralin or danthron in cultured human cells. *J. Invest. Dermatol.* **1982**, *79*, 18–22.

(51) Reichert, U.; Jacques, Y.; Grangeret, M.; Schmidt, R. Antirespiratory and antiproliferative activity of anthralin in cultured human keratinocytes. *J. Invest. Dermatol.* **1985**, *84*, 130–134.

(52) Remitz, A.; Kariniemi, A.-L.; Lehtonen, E.; Nordling, S. Inhibition of proliferation of HeLa cells by dithranol (anthralin) and 10-butyryl dithranol (butantrone). *Br. J. Dermatol.* **1989**, *120*, 525–531.
(53) Bonnekoh, B.; Farkas, B.; Geisel, J.; Mahrle, G. Lactate dehydrogenase release as an indicator of dithranol-induced membrane injury in cultured human keratinocytes. *Arch. Dermatol. Res.* **1990**, *282*, 325–329.
(54) Lassus, A.; Forsstrom, S. A dimethoxynaphthalene derivative (RS-43179 gel) compared with 0.025% flucinolone acetonide gel in the treatment of psoriasis. *Br. J. Dermatol.* **1985**, *113*, 103–106.
(55) Burdon, R. H.; Gill, V.; Rice-Evans, C. Cell proliferation and oxidative stress. *Free Radical Res. Commun.* **1989**, *7*, 149–159.
(56) Hsieh, G. C.; Acosta, D. Dithranol-induced cytotoxicity in primary cultures of rat epidermal keratinocytes. I. The role of reactive oxygen species. *Toxicol. Appl. Pharmacol.* **1991**, *107*, 16–26.
(57) Imlay, J. A.; Linn, S. DNA damage and oxygen radical toxicity. *Science* **1988**, *240*, 1302–1309.
(58) Halliwell, B.; Aruoma, O. I. DNA damage by oxygen-derived species. Its mechanism and measurement in mammalian systems. *FEBS Lett.* **1991**, *281*, 9–19.
(59) Kragballe, K.; Desjarlais, L.; Voorhees, J. J. Leukotrienes B_4, C_4 and D_4 stimulate DNA synthesis in cultured human epidermal keratinocytes. *Br. J. Dermatol.* **1985**, *113*, 43–52.
(60) von Braun, J. Degradation of α-bromo acids to aldehydes and ketones. *Ber. Dtsch. Chem. Ges.* **1934**, *67*, 218–225.
(61) Clark, E. R.; Robson, R. D. Œstrogenic carboxylic acids. Part II. Open chain analogues of doisynolic acid. *J. Chem. Soc.* **1959**, 3714–3722.
(62) Corey, E. J.; Suggs, J. W. Pyridinium chlorochromate. An efficient reagent for oxidation of primary and secondary alcohols to carbonyl compounds. *Tetrahedron Lett.* **1975**, *31*, 2647–2650.
(63) Rapoport, H.; Campion, J. E. The synthesis of 2,3,4-trimethoxybenzcyclohepten-6-one. *J. Am. Chem. Soc.* **1951**, *73*, 2239–2241.
(64) Auterhoff, H.; Scherff, F. C. Dianthrones of pharmaceutically interesting hydroxyanthraquinones. *Arch. Pharm. (Weinheim, Ger.)* **1960**, *293*, 918–925.
(65) Unger, S. H.; Cook, J. R.; Hollenberg, J. S. Simple procedure for determining octanol-aqueous partition, distribution, and ionization coefficients by reversed-phase high-pressure liquid chromatography. *J. Pharm. Sci.* **1978**, *67*, 1364–1367.