Antipsoriatic Anthrones with Modulated Redox Properties. 5. Potent Inhibition of Human Keratinocyte Growth, Induction of Keratinocyte Differentiation, and Reduced Membrane Damage by Novel 10-Arylacetyl-1,8-dihydroxy-9(10*H*)-anthracenones

Klaus Müller,*,‡ Hans Reindl,§ and Klaus Breu§

Institut für Pharmazeutische Chemie, Westfälische Wilhelms−Universität Münster, Hittorfstrasse 58-62, D-48149 Münster, Germany, and Institut für Pharmazie, Pharmazeutische Chemie I, Universität Regensburg, D-93040 Regensburg, Germany

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The synthesis and structure−activity relationships (SARs) of a series of novel 10-arylacetyl-1,8-dihydroxy-9(10*H*)-anthracenones are described. Acylation of anthralin with either the appropriate arylacetyl chlorides or arylacetic acids in the presence of pyridine or via the coupling agent dicyclohexylcarbodiimide (DCC), respectively, furnished this structural class of antipsoriatic agents. Potential antipsoriatic activity was evaluated in complementary assays specifically addressed to three important aspects of psoriasis. First, several compounds were identified which are equally potent as inhibitors of human keratinocyte growth as the antipsoriatic agent anthralin. Furthermore, improved ratio of antiproliferative activity to cytotoxicity is demonstrated by the reduced potential of the novel analogues to induce membrane damage, which is a benefit of their reduced ability to generate oxygen radicals as documented by deoxyribose degradation. Second, analogue **3o** bearing a hydroxamate functional group was also a highly potent inhibitor of LTB_4 biosynthesis in addition to its excellent antiproliferative activity. SARs of these inhibitors of both keratinocyte growth and LTB_4 biosynthesis with respect to the nature of the *para*-substitution in the 10-phenylacetyl side chain are discussed. Third, the compounds were also evaluated for their ability to induce the formation of cornified envelope protein in keratinocytes. Cross-linking of cellular protein as a marker of terminal differentiation of keratinocytes was observed for many 10-arylacetyl analogues at concentrations required to arrest cell growth. This newly uncovered activity of the novel anthracenones suggests antipsoriatic potential with respect to disturbance of keratinocyte differentiation, in addition to hyperproliferative and inflammatory aspects of psoriasis.

Introduction

Psoriasis is a common skin disease with a prevalence of 1−2% of the population in northern Europe and North America.[1] Dominant and interdependent features of the disease are epidermal hyperproliferation, disturbed keratinocyte differentiation, and inflammation of the dermis and epidermis.[2] Current treatments for psoriasis may be topical or systemic.[1,3] Indications for systemic treatment are failure to respond to topical treatment and severe or life-threatening forms of psoriasis.[4] More recently, vitamin D analogues and vitamin A analogues have been added to the traditional topical therapy, but these agents serve only to abate the disease.[5] Yet anthracenones such as anthralin (**1**, dithranol) clear the rash totally.[6] Indeed, of all antipsoriatic agents, anthralin has proven to be the most remarkably consistent and time-honored drug for treating psoriasis.[7] Because of the undesirable side effects such as severe inflammation of nonaffected skin associated with this topical agent, sometimes successful courses of treatment must even be halted. Perhaps the best solution of this problem would be development of structural analogues in which antipsoriatic efficacy is retained while proinflammatory effects are minimized.[8]

The methylene moiety at C-10 of anthralin has been recognized as a key site of the formation of oxygen radicals,[9] which play a fundamental role in the induction of skin inflammation. Moreover, in addition to directly damaging cellular components of the skin, oxygen radicals are responsible for the induction of inflammatory cytokines which, in turn, contributes to the dermal toxicity of anthralin.[10] Therefore, our strategy to overcome this problem was to modulate the oxygen-radical generating intensity by modifying the critical 10-position of the pharmacophore. Since our fundamental work with anthracenone-based antipsoriatics established that the 1,8-dihydroxy substitution pattern of anthralin is required for potent antiproliferative activity,[11] we decided to retain this feature in our compounds. In our earlier report, we have established 10-substituted derivatives as antipsoriatic anthracenones with modulated redox properties, and we demonstrated their inhibitory activity against 5-lipoxygenase.[12]

In our continuing investigation of structure−activity relationships (SARs) of the anthracenone class of antipsoriatic agents, we further studied the biological properties of 10-substituted congeners of anthralin. In this paper, we report the synthesis of novel 10-arylacetyl

* To whom correspondence should be addressed. Tel: +49 251-8333324. Fax: +49 251-8332144. E-mail: kmuller@uni-muenster.de.
‡ Universität Münster.
§ Universität Regensburg.

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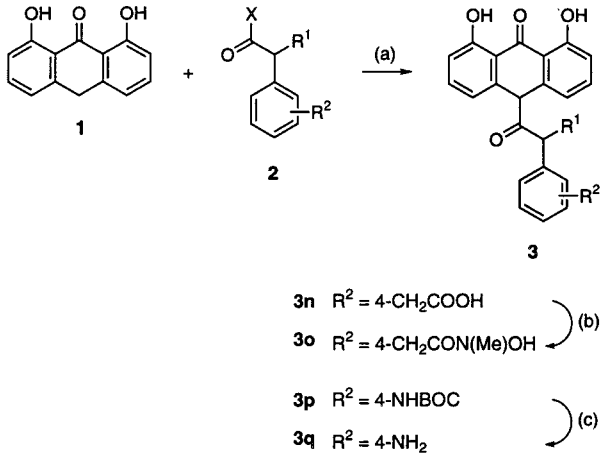
Scheme 1[a]



[a] Reagents: (a) method A, X = Cl: pyridine, THF, N_2; method B, X = OH: DCC, pyridine, THF, N_2; (b) NH(Me)OH·HCl, EDC, DMF, N_2; (c) THF, trifluoroacetic acid. R^1 and R^2 are defined in Table 1.

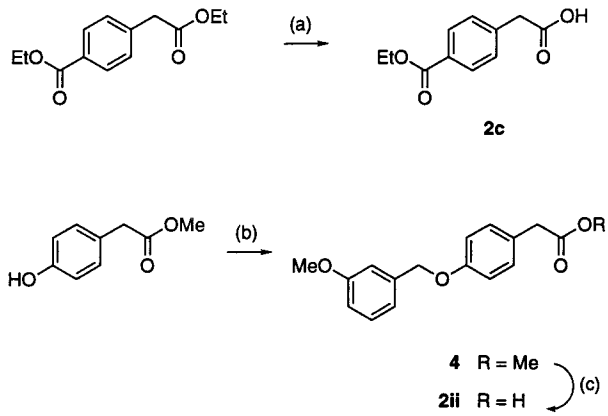
analogues and the assessment of their potential value as antipsoriatic agents in complementary assays specifically addressed to three important aspects of psoriasis. Thus, in addition to their leukotriene B_4 (LTB_4) inhibitory action as a measure of their antiinflammatory properties, we also evaluated their antiproliferative activity against the growth of HaCaT keratinocytes and we describe their potential to induce differentiation of human keratinocytes. Finally, we examined their cytotoxicity in terms of membrane-damaging effects.

Chemistry

Some of the required *para*-substituted 10-phenylacetyl analogues were available from previous work, and these are referenced in Table 1. The novel 10-phenylacetyl analogues were prepared by reaction of **1** with appropriate phenylacetyl chlorides in the presence of pyridine (Scheme 1, method A, X = Cl), where acylation takes place at the C-10 position via the carbanion.[13] The required phenylacetyl chlorides were prepared from the corresponding phenylacetic acids **2** according to literature procedures.[14,15] In an alternative method (method B, X = OH), phenylacetic acids **2** were directly introduced onto the 10-position of **1** via the coupling agent dicyclohexylcarbodiimide (DCC).[16] For easier workup in the preparation of **3n**, ethyl *N*,*N*-dimethylaminopropylcarbodiimide (EDC) was used in place of DCC. EDC was also used to obtain the hydroxamate **3o** from **3n** and *N*-methylhydroxylamine hydrochloride. For the preparation of **3q**, the amino group of 4-aminophenylacetic acid was protected as the *tert*-butoxycarbonyl (BOC) derivative **2p**. Reaction of **2p** with **1** in the presence of DCC produced analogue **3p**, which upon deprotection with trifluoroacetic acid gave **3q**.

The other starting arylacetic acids were either commercial products or prepared according to the literature. Scheme 2 shows the preparation of 4-ethoxycarbonyl analogue **2c** (for the preparation of **3c**), which was obtained from the appropriate ethyl phenylacetate by selective saponification of the aliphatic ester group by analogy to the method of Maillard.[17] Benzyloxyphenylacetic acid **2ii** (for the preparation of **3ii**) was produced from methyl 4-hydroxyphenylacetate by reaction with

Scheme 2[a]



[a] Reagents: (a) Na, EtOH, H_2O, rt, 12 h; (b) K_2CO_3, 3-MeOBnCl, KI, acetone, Δ, 12 h; (c) 6 N NaOH, Δ, 4 h.

3-methoxybenzyl chloride followed by saponification of the resulting **4**. Compounds **3kk**, **3mm**, **3nn**, and **3tt** were prepared from the antiinflammatory arylacetic acids diclofenac, ibuprofen, ketoprofen, and indomethacin, respectively.

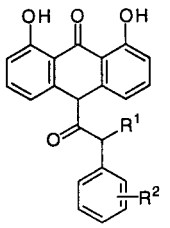
Biological Evaluation and Discussion

The structures of the 10-arylacetyl substituted anthracenones are listed in Table 1, together with relevant biological properties. The biological assay procedures used were exactly as those described in our previous studies.[12,18,19] Thus, modulated redox properties in terms of hydroxyl-radical generation were studied using the deoxyribose assay.[12] The release of malondialdehyde (MDA) is indicative of hydroxyl-radical generation. Anthralin (**1**) and compound **3a** in Table 1 are the parent anthracenone and the unsubstituted 10-phenylacetyl analogue (R = H), respectively. Comparison documents the positive effect for the phenylacetyl substituent, which dramatically decreases hydroxyl-radical formation.[12] Table 1 shows that, with the exception of a few compounds such as **3p** and **3q**, generation of hydroxyl radicals is markedly reduced or not significantly different with respect to controls.

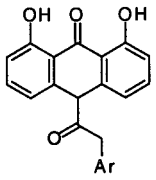
Inhibition of Keratinocyte Growth and LTB_4 Biosynthesis. The immortalized keratinocyte line HaCaT[20] was used to mimic the hyperproliferative epidermis found in psoriasis, as antiproliferative action in cell cultures may be critical in the management of the proliferative component of psoriasis. Proliferation of the keratinocytes was determined directly by counting the dispersed cells under a phase-contrast microscope after 48 h of treatment. Inhibition of LTB_4 biosynthesis by the novel compounds was determined by measuring the production of LTB_4 in bovine polymorphonuclear leukocytes.

Results from the HaCaT keratinocyte proliferation assay show that the phenylacetyl analogue **3a** also retains the antiproliferative activity of the parent **1**, although the potency is somewhat reduced. Compounds **3b**−**m** compare the effects of a number of electron-withdrawing groups at the *para*-position. It can be seen that, compared with hydrogen, no marked improvements are obtained. Although the *para*-bromo derivative **3k** is a potent inhibitor of LTB_4 biosynthesis, inhibition against HaCaT cell growth is decreased as compared to **1** and **3a**.

Table 1. Deoxyribose Degradation, Antiproliferative Activity, and Cytotoxicity against HaCaT Cells and Inhibition of LTB_4 Biosynthesis in Bovine PMNL by 10-Arylacetyl-1,8-dihydroxy-9(10*H*)-anthracenones



3a–3oo



3pp–3tt

compd	R^1	R^2	DD (•OH)[a]	AA IC_{50} (μM)[b]	LDH (mU)[c]	LTB_4 IC_{50} (μM)[d]
3a[e]	H	H	<0.3	1.9	ND	11
3b	H	4-CO_2Me	<0.3	1.8	164	12
3c	H	4-CO_2Et	<0.3	1.3	ND	6
3d	H	4-CN	ND	3.2	ND	9
3e[e]	H	4-NO_2	<0.3	4.7	179	7
3f	H	4-CF_3	ND	1.6	ND	11
3g	H	4-F	<0.3	2.6	ND	11
3h	H	4-Cl	<0.3	2.2	ND	1
3i	H	3,4-Cl_2	ND	1.2	ND	9
3k	H	4-Br	<0.3	2.9	ND	0.7
3l	H	4-I	<0.3	3.1	ND	1.5
3m	H	4-Ph	<0.3	2.2	ND	7
3n	H	4-CH_2COOH	0.93 ± 0.09[f]	1.7	ND	3
3o	H	4-CH_2CON(Me)OH	0.51 ± 0.08[f]	0.7	199	0.05
3p	H	4-NHBOC	0.97 ± 0.13[f]	1.8	ND	2
3q	H	4-NH_2	1.43 ± 0.10[f]	1.2	ND	1
3r	H	4-NMe_2	ND	1.3	ND	6
3s[e]	H	4-OH	0.36 ± 0.07[f]	1.7	173	14
3t[e]	H	3,5-(*t*-Bu)$_2$-4-OH	<0.3	3.0	199	10
3u	H	4-SMe	ND	1.6	ND	6
3v	H	4-Me	<0.3	0.7	145[g]	0.6
3w[e]	H	4-OMe	<0.3	1.1	143[g]	0.5
3x	H	2-OMe	<0.3	1.6	125[g]	17
3y	H	3-OMe	<0.3	2.7	ND	3
3z	H	3-OH-4-OMe	ND	1.9	ND	8
3aa	H	3,4-$(OMe)_2$	0.86 ± 0.20[f]	4.2	ND	22
3bb	H	3,4-OCH_2O	<0.3	3.3	ND	1
3cc	H	4-OEt	<0.3	1.7	142[g]	1
3dd	H	4-OPr	<0.3	1.6	132[g]	1
3ee	H	4-O-*i*-Pr	<0.3	1.8	ND	15
3ff	H	4-OBu	<0.3	1.2	ND	1.5
3gg[e]	H	4-OBn	<0.3	2.4	202	0.6
3hh	H	4-O(4-MeOBn)	<0.3	3.2	ND	1.5
3ii	H	4-O(3-MeOBn)	<0.3	1.0	159	1
3kk	H	2-NH(Ph-2,6-Cl_2)	0.45 ± 0.01[f]	1.3	247	3
3ll	Me[h]	H	<0.3	1.0	ND	18
3mm	Me[h]	4-*i*-Bu	<0.3	3.5	ND	16
3nn	Me[h]	4-Bz	<0.3	0.9	191	9
3oo	Ph	H	<0.3	1.1	ND	7
		Ar				
3pp		1-naphthyl	<0.3	2.6	ND	3
3qq		2-naphthyl	<0.3	2.9	ND	3
3rr		cyclohexyl	<0.3	1.7	ND	13
3ss		3-indolyl	<0.3	0.8	174	9
3tt		3-indolyl of indomethacin	ND	3.9	ND	15
anthralin (**1**)		2.89 ± 0.14[f]	0.7	294	37	

[a] 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; values are significantly different with respect to control, $P < 0.01$). [b] Antiproliferative activity against HaCaT cells. Inhibition of cell growth was significantly different with respect to control, $N = 3$, $P < 0.01$. [c] Activity of LDH (mU) release in HaCaT cells after treatment with 2 μM test compound ($N = 3$, SD < 10%). [d] Inhibition of LTB_4 biosynthesis in bovine polymorphonuclear leukocytes. Inhibition was significantly different with respect to control, $N = 3$ or more, $P < 0.01$. Nordihydroguaiaretic acid (NDGA) was used as a standard ($IC_{50} = 0.4$ μM). [e] Ref 12. [f] Values are significantly different with respect to vehicle control. [g] Values are not significantly different with respect to vehicle control. [h] Racemate. ND = not determined.

Preliminary experiments indicated that addition of a *para*-methoxy group (**3w**) to the unsubstituted **3a** greatly enhanced inhibitory action against 5-lipoxygenase.[12] Moreover, as can be seen in Table 1, the antiproliferative activity of **1** is retained. By contrast, changing the methoxy group of **3w** to other electron-donating *para*-groups (**3p**−**u**) is moderately disadvantageous for both antiproliferative and LTB_4 inhibitory activity. The exception is the *para*-methyl analogue **3v** which shares the same excellent antiproliferative activity with **1** and the LTB_4 inhibitory potency with **3w**.

The importance of the position of the methoxy group on the phenyl ring is investigated by comparing analogues **3w**−**y**. Moving the methoxy group to the *ortho*-

or *meta*-position as in **3x** and **3y** results in a 34- and 6-fold decrease in inhibition of LTB_4 biosynthesis, respectively. Also, inhibition of keratinocyte growth is decreased. If, however, the methoxy group is moved away from the phenylacetyl group by insertion of a benzyl group, slightly better potency is observed for the *meta*-methoxy analogue **3ii** as compared to the *para*-methoxy analogue **3hh**.

Analogues **3z** and **3aa** explore the effects of an additional hydroxyl or an extra methoxy group, respectively. In both cases, potency is decreased as compared to **3w**. Incorporating the methoxy group in the cyclic 3,4-methylenedioxy substituent (**3bb**), which maintains the electronic character of the acyclic analogue **3aa** but introduces additional structural rigidity, gives satisfactory potency only in the LTB_4 assay.

The series **3w**,**cc**−**ff** further explores the utility of 4-alkoxy substitution, covering alkyl groups from methyl to butyl. No further increase in potency is seen at longer chain length (**3cc**,**dd**,**ff**) or a branched chain (**3ee**). While potent inhibition of LTB_4 biosynthesis is maintained with a benzyloxy group (**3gg**), growth-inhibitory effects are decreased.

Furthermore, incorporation of arylacyl moieties of antiinflammatory agents such as diclofenac (**3kk**), ibuprofen (**3mm**), ketoprofen (**3nn**), and indomethacin (**3tt**) into hybridized structures decreases LTB_4 inhibitory action as compared with that of the most active compounds (**3k**,**o**,**v**,**w**,**gg**) of this series. However, except for the lipophilic ibuprofen and indomethacin derivatives, potent inhibition of keratinocyte growth is observed.

Since the potential of generating potent inhibitors of leukotriene biosynthesis by incorporating a hydroxamate moiety has been described,[21,22] and in particular *N*-methylhydroxamate proved to be useful in 2-substituted anthracenones,[23] we also explored the utility of such a functional group. Indeed, substitution of the *para*-position of the phenyl ring with an *N*-hydroxy-*N*-methylacetamide group (**3o**) resulted in the most potent inhibitor of LTB_4 biosynthesis within the entire series of antipsoriatic anthracenones described so far. With an IC_{50} value in the nanomolar range, the potency of **3o** is comparable to those of the most potent leukotriene biosynthesis inhibitors.[24] In addition, this analogue retains the high antiproliferative activity of the parent **1**.

Finally, we were also interested in seeing if replacement of the terminal phenyl ring of the C-10 substituent with a nonaromatic, annelated, or heterocyclic ring led to active compounds. With respect to the unsubstituted phenylacetyl analogue (**3a**), replacement with a naphthalene system (**3pp**,**qq**) or cyclohexane ring (**3rr**) does not result in dramatic changes in activity, whereas the antiproliferative activity of the 3-indole analogue **3ss** is significantly increased.

Among the variety of diseases in which the leukotrienes, the products of the 5-lipoxygenase pathway of arachidonic acid metabolism, play a symptomatic or causative role is psoriasis.[25] LTB_4 regulates accumulation of polymorphonuclear leukocytes,[26] and application of LTB_4 induces changes similar to those found in psoriasis.[27] Although still controversial,[24] there is substantial evidence that LTB_4 may play a significant role in the amplification of many inflammatory disease states including psoriasis.[28] Accordingly, inhibition of the enzymes or antagonism of leukotriene biosynthesis is explored as a potentially viable approach to antipsoriatic therapy.[29−31] An antipsoriatic agent that inhibits the biosynthesis of LTB_4 *in addition* to growth-inhibitory effects on keratinocytes may reflect improved antipsoriatic activity with respect to the inflammatory component of psoriasis.

LTB_4 can also effect keratinocyte proliferation,[32,33] and cultured HaCaT keratinocytes express the 5-lipoxygenase gene.[34] Consequently, the better antiproliferative activity observed with compounds **3o** and **3v** may be related to the more potent LTB_4 inhibitory activity also seen with those compounds. However, based upon the weaker results obtained with the potent LTB_4 biosynthesis inhibitors **3k** and **3gg** in the keratinocyte assay, it is unclear whether inhibition of LTB_4 biosynthesis actually plays a role in the antiproliferative activity of these compounds. Therefore, the antiproliferative activity of the *para*-substituted analogues and their ability to inhibit LTB_4 biosynthesis was compared. Plots of the log IC_{50} for inhibition of HaCaT cell growth versus log IC_{50} for inhibition of LTB_4 biosynthesis (data not shown) do not support any relationship between the effects of the compounds in the two kinds of assays ($r = 0.06$).

Membrane Damage. Furthermore, keratinocytes were also tested for their susceptibility for the action of the compounds on plasma membrane integrity. Cytotoxicity of the cell cultures was assessed by the activity of lactate dehydrogenase (LDH) released into the culture medium,[19] which is commonly used as an indicator of plasma membrane damage. As a result of its potential to generate oxygen radicals, parent **1** is an inducer of lipid peroxidation in biological membranes.[35] In contrast to **1**, which shows a 2-fold increase in LDH activity as compared to controls, LDH release after treatment of HaCaT cells with the most potent inhibitors of cell growth, **3o** and **3v**, is markedly decreased or does not exceed the control values, respectively.

Induction of Keratinocyte Differentiation. Besides increased inflammation of the skin and keratinocyte hyperproliferation, abnormal keratinocyte differentiation is an important pathogenic factor of psoriasis.[2] Since differentiation is an irreversible process, killing aberrant cells which are then lost by sloughing, agents that induce this process can be used in the treatment of hyperproliferative conditions. HaCaT cells, although immortalized and genetically abnormal, share many features of differentiation with normal keratinocytes,[36] and the highly preserved epidermal characteristics make this cell line an excellent candidate for studying external modulators of epidermal differentiation.[37] Keratinocytes change in cell morphology, increase in cell size, and change in the proportion of cells that increase the amount of cornified envelope protein, a major structural component generated as a result of the process of keratinocyte differentiation.[38] The cornified envelopes are composed predominantly of an array of cross-linked proteins.[39] To further investigate the antipsoriatic potential of the novel anthracenones, we examined their ability to induce the formation of cross-linked protein envelopes. The data of representative anthracenones are presented in Table 2.

Table 2. Induction of Keratinocyte Differentiation by Selected Anthracenones and Standard Drugs

	cross-linked envelope assay[a] (μg cross-linked protein/mg protein)		
compd	1 μM	5 μM	10 μM
3g	0.6 ± 0.2	2.4 ± 0.3[b]	3.5 ± 0.1[b]
3m	1.8 ± 0.6	3.8 ± 0.5[b]	4.6 ± 0.5[b]
3o	2.8 ± 0.2[b]	5.5 ± 0.2[b]	6.2 ± 0.5[b]
3q	0.0 ± 1.9	6.0 ± 0.3[b]	6.6 ± 0.3[b]
3s	2.5 ± 0.9	5.1 ± 0.3[b]	6.2 ± 0.2[b]
3v	2.1 ± 0.7	4.2 ± 0.4[b]	5.1 ± 0.1[b]
3w	2.3 ± 0.3[b]	5.3 ± 0.2[b]	5.8 ± 0.5[b]
3ff	2.5 ± 0.2[b]	3.4 ± 0.4[b]	3.4 ± 0.2[b]
3ss	1.8 ± 0.4	4.9 ± 0.3[b]	6.2 ± 0.4[b]
anthralin (**1**)	3.7 ± 1.8[b]	4.9 ± 0.8[b]	5.8 ± 1.4[b]
danthron	0.6 ± 1.8	−0.1 ± 0.7	0.0 ± 1.0
9(10*H*)-anthracenone	0.5 ± 1.2	0.3 ± 0.6	0.8 ± 1.8
10-Bn-anthralin	−1.0 ± 0.3	−0.8 ± 0.1	0.9 ± 0.6

[a] Differences of the amounts of cross-linked protein as a measure of HaCaT keratinocyte differentiation at indicated concentrations of test compounds and vehicle control. Results are the means ± SEM of three independent experiments. [b] Values are significantly different with respect to vehicle control ($P < 0.05$, Student's *t*-test).

Anthralin is able to induce cross-linked protein as a measure of terminal differentiation in the keratinocyte cell line HaCaT in a concentration-dependent manner. By contrast, the unsubstituted 9(10*H*)-anthracenone and the anthralin metabolite danthron (1,8-dihydroxy-9,10-anthracenedione), which are both therapeutically inactive and do not arrest keratinocyte growth,[11] are not able to induce keratinocyte differentiation. Also, alkyl substitution in the 10-position of anthralin as in 10-benzylanthralin[12] does not exhibit appreciable activity within the concentration range, indicating that structural features necessary for cross-linking do not reside necessarily in the anthralin chromophore. On the other hand, the novel 10-arylacetyl analogues are all able to induce significant cross-linking, with compounds **3o**, **3w**, and **3ff** doing so down to the lowest concentration of 1 μM. These compounds also effect growth arrest at concentrations required to induce protein cross-linking, demonstrating a causality between these two effects. In contrast to parent **1** which induces membrane damage, nonspecific toxicity cannot explain the changes caused by the novel anthracenones insofar as treated keratinocytes had normal membrane function (assessed by LDH release) and synthesized protein faster than control cells. While for other 10-arylacetyl analogues growth inhibition may occur at slightly lower concentrations than those required to induce differentiation, comparatively small differences in cross-linking potency at the concentrations used make a consistent SAR correlation within this series of analogues difficult to derive. A somewhat higher cross-linking potency is generally found with more hydrophilic compounds (log *P* values are given in Table 3).

The results presented herein demonstrate that 10-arylacetylanthracenones not only inhibit keratinocyte proliferation but also induce their differentiation. While growth arrest may be a necessary consequence of keratinocyte differentiation,[40] growth arrest does not necessarily result in the onset of differentiation.[41,42] Terminal differentiation of keratinocytes is documented by the ability of the anthracenones to cross-link proteins into cornified envelope-like structures, resulting in withdrawal of cells from the cell cycle, which is many times faster in psoriasis than normal.[2]

In conclusion, the present study has confirmed that arylacetyl substituents at the 10-position of **1** provide analogues with diminished hydroxyl-radical formation and improved inhibitory action against LTB_4 biosynthesis. Furthermore, appropriate *para*-substitution in the 10-phenylacetyl side chain can modify **1** such that it retains potent antiproliferative activity combined with reduced membrane damage and the ability to induce terminal differentiation of keratinocytes. However, comparison of the ability of the *para*-substituted analogues to inhibit LTB_4 biosynthesis and their ability to arrest keratinocyte growth does not support any relationship between the effects of the analogues in the two kinds of assays. The most potent analogues, **3o** and **3v**, show an attractive profile of biological activity and compare favorably with **1** as potential alternatives for antipsoriatic therapy.

Experimental Section

Melting points were determined with a Büchi 510 melting point apparatus and are uncorrected. Lipophilicities, as indicated by log *P* values, were determined by a standard HPLC procedure.[43] Chromatography refers to column chromatography on silica gel (E. Merck, 70−230 mesh); eluants are given in Table 3. ^{1}H NMR spectra were recorded with a Varian EM 390 (90 MHz) or 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. 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). Data were recorded on a MacLab data acquisition system (WissTech, Germany) and analysis was performed with the software Peaks on an Apple Macintosh computer.

Preparation of Phenylacetic Acids. Phenylacetic acids **2** (Scheme 1, X = OH) were commercial products or prepared by following standard literature procedures: **2b**,[17] **2d**,[44] **2p**,[45] **2z**,[46] **2cc**−**ff**,[47] **2hh**.[48]

4-Ethoxycarbonylphenylacetic Acid (2c). To a solution of Na (345 mg, 15 mmol) in absolute EtOH (40 mL) was added ethyl 4-ethoxycarbonylphenylacetate[49] (4.00 g, 16.9 mmol). The solution was treated with absolute EtOH (100 mL) and then with water (0.28 mL). Then it was stirred at room temperature for 12 h, and the resulting precipitate was filtered by suction and dissolved in water. The solution was adjusted to pH 1 with 2 N HCl, and the precipitate was extracted with EtOAc (3 × 50 mL). The organic phase was dried over Na_2SO_4 and then concentrated, and small amounts of hexane were added to induce precipitation of white crystals: 51% yield; mp 101 °C; FTIR 2983, 1721, 1701 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.92, 7.33 (dd, J = 9 Hz, 4H), 4.42−4.19 (q, J = 7 Hz, 2H), 3.67 (s, 2H), 1.30 (t, J = 7 Hz, 3H); MS m/z = 208 (20, M^+), 163 (100). Anal. ($C_{11}H_{12}O_4$) C, H.

Methyl 4-(3-Methoxyphenyl)methoxyphenylacetate (4). To a solution of methyl 4-hydroxyphenylacetate (3 g, 18 mmol) in absolute acetone (50 mL) were added dry K_2CO_3 (13.8 g, 10 mmol), 3-methoxybenzyl chloride (4.37 g, 28 mmol), and a catalytic amount of KI. The mixture was heated to reflux under vigorous stirring for 12 h. Then it was filtered, the filtrate was evaporated, and the residue was purified by chromatography using CH_2Cl_2/hexane (9/1) to yield a pale yellow fluid: 60% yield; bp 201−203 °C/1600 Pa; FTIR 1736 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.31−6.74 (m, 8H), 4.87 (s, 2H), 3.78 (s, 3H), 3.65 (s, 3H), 3.53 (s, 2H); MS m/z = 286 (17, M^+), 121 (100). Anal. ($C_{17}H_{18}O_4$) C, H.

Table 3. Chemical Data of 10-Arylacetyl-1,8-dihydroxy-9(10*H*)-anthracenones

compd	log *P*[a]	formula[b]	method[c]	mp (°C)	% yield[d]	solvent[e]
3b	4.07	$C_{24}H_{18}O_6$	A	197−198	20	MC/E (24/1)
3c	4.34	$C_{25}H_{20}O_6$	A	161−162	18	MC/E (9/1)
3d	3.58	$C_{23}H_{15}NO_4$	B	188−190[f]	36	MC/E (9/1)[g]
3f	4.40	$C_{23}H_{15}F_3O_4$	B	165−167	29	MC/H (8/2)[h]
3g	4.11	$C_{22}H_{15}FO_4$	A	178−180	39	MC
3h	4.49	$C_{22}H_{15}ClO_4$	A	163−165	34	MC/H (8/2)[i]
3i	4.74	$C_{22}H_{14}Cl_2O_4$	B	162−163	26	MC/PE (7/3)[j]
3k	4.60	$C_{22}H_{15}BrO_4$	A	179−181	30	MC/H (8/2)[i]
3l	4.80	$C_{22}H_{15}IO_4$	A	190−191	34	MC/H (8/2)
3m	5.02	$C_{28}H_{20}O_4$	B	188[f]	22	MC/PE (7/3)[k]
3n	2.84	$C_{24}H_{18}O_6$	C	197[f]	16	MC/M (9/1)[g]
3o	3.02	$C_{25}H_{21}NO_6$	c	150[f]	5	MC/M (98/2)
3p	4.17	$C_{27}H_{25}NO_6$	B	204−206[f]	38	MC/E (19/1)
3q	3.29	$C_{22}H_{17}NO_4$	c	163−164	67	*l*
3r	4.42	$C_{24}H_{21}NO_4$	B	178[f]	31	MC/EE (99/1)[k]
3u	4.42	$C_{23}H_{18}O_4S$	B	167[f]	28	*i*
3v	4.54	$C_{23}H_{18}O_4$	B	152−154	18	MC/H (8/2)[h]
3x	4.20	$C_{23}H_{18}O_5$	A	178−179	31	MC/H (17/3)
3y	4.16	$C_{23}H_{18}O_5$	A	140	21	MC/H (17/3)
3z	3.41	$C_{23}H_{18}O_6$	B	138−141	26	MC/E (9/1)[i]
3aa	3.77	$C_{24}H_{20}O_6$	A	165−166	23	MC/E (19/1)
3bb	4.06	$C_{23}H_{16}O_6$	A	182	25	MC/H (7/3)
3cc	4.51	$C_{24}H_{20}O_5$	A	162	21	MC/H (8/2)
3dd	4.97	$C_{25}H_{22}O_5$	A	135−136	25	MC/H (17/3)
3ee	4.59	$C_{25}H_{22}O_5$	A	143	24	MC
3ff	5.41	$C_{26}H_{24}O_5$	A	114	34	MC/H (9/1)
3hh	4.91	$C_{30}H_{24}O_6$	A	159	24	MC/H (8/2)
3ii	5.19	$C_{30}H_{24}O_6$	A	147−149	28	MC/H (19/1)
3kk	3.91	$C_{28}H_{19}Cl_2NO_4$	B	183−184	6	MC/PE (7/3)[m]
3ll	4.43	$C_{23}H_{18}O_4$	A	184−185	13	MC/H (7/3)
3mm	5.57	$C_{27}H_{26}O_4$	B	179−181	9	MC/PE (6/4)[n]
3nn	4.46	$C_{30}H_{22}O_5$	B	128−133	9	MC[o]
3oo	4.60	$C_{28}H_{20}O_4$	B	193[f]	13	MC/PE (1/1)[j]
3pp	4.61	$C_{26}H_{18}O_4$	B	207[f]	20	MC/PE (1/1)[j]
3qq	4.71	$C_{26}H_{18}O_4$	B	159−162	20	MC/PE (6/4)[k]
3rr	4.97	$C_{22}H_{22}O_4$	B	139−142	21	EE/PE (3/7)[j]
3ss	3.77	$C_{24}H_{17}NO_4$	B	193[f]	28	MC[o]
3tt	5.17	$C_{33}H_{24}ClNO_6$	B	148−150	10	MC[o]

[a] Experimentally determined partition coefficients.[43] [b] All new compounds displayed ^{1}H NMR, FTIR, UV, and MS spectra consistent with the assigned structure. Elemental analyses were within ±0.4% of calculated values. [c] See Experimental Section. [d] Yields have not been optimized. [e] Chromatography solvent (vol %): A = acetic acid; E = ether; EE = ethyl acetate; H = hexane; M = methanol; MC = methylene chloride; PE = petroleum ether. [f] Decomposition. [g] Recrystallized from benzene/THF (8/2). [h] Recrystallized from benzene/hexane (7/3). [i] Recrystallized from benzene. [j] Recrystallized from benzene/petroleum ether (1/1). [k] Recrystallized from benzene/petroleum ether (6/4). [l] Recrystallized from benzene/ethanol (8/2). [m] Recrystallized from methylene chloride/petroleum ether (1/1). [n] Recrystallized from benzene/petroleum ether (2/8). [o] Recrystallized from benzene/petroleum ether (8/2).

4-(3-Methoxyphenyl)methoxyphenylacetic Acid (2ii). A suspension of **4** (2.29 g, 8 mmol) in 6 N NaOH (30 mL) was heated to reflux for 4 h. The mixture was cooled and neutralized with 6 N HCl, and the resulting precipitate was extracted with EtOAc (3 × 30 mL). The organic phase was dried over Na_2SO_4 and then concentrated. Petroleum ether (40−60) was added to induce precipitation of white crystals: 66% yield; mp 86 °C; FTIR 1717 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 12.24 (br s, 1H), 7.40−6.82 (m, 8H), 4.89 (s, 2H), 3.75 (s, 3H), 3.49 (s, 2H); MS m/z = 272 (17, M^+), 121 (100). Anal. ($C_{16}H_{16}O_4$) C, H.

Preparation of Phenylacetyl Chlorides. Phenylacetyl chlorides **2** (Scheme 1, X = Cl) were prepared from the corresponding phenylacetic acids by following standard literature procedures.[14,15] In most cases the crude products were used in the subsequent acylation steps.

General Procedure for the Preparation of 10-Arylacetyl-1,8-dihydroxy-9(10*H*)-anthracenones. Method A. 10-[2-(4-Bromophenyl)-1-oxoethyl]-1,8-dihydroxy-9(10*H*)-anthracenone (3k). To a solution of anthralin[50] (**1**; 1.00 g, 4.42 mmol) in absolute THF (30 mL) and dry pyridine (1.0 mL, 12.70 mmol) was added dropwise a solution of 4-bromophenylacetyl chloride (**2k**, X = Cl; 1.03 g, 4.42 mmol) in absolute THF (10 mL) under N_2. The reaction mixture was stirred at room temperature for 4 h, filtered, and the filtrate was evaporated. The residue was purified by chromatography and recrystallized from benzene to afford **3k** as yellow needles (Table 3): FTIR 1711 (COOH), 1630 cm^{-1} (CO···HO); ^{1}H NMR ($CDCl_3$) δ 12.27 (2H), 7.55−6.58 (m, 10H), 5.30 (s, 1H), 3.27 (s, 2H); MS m/z 424 (3.5), 422 (3.8). Anal. ($C_{22}H_{15}BrO_4$) C, H.

Method B. 1,8-Dihydroxy-10-[2-(4-methylphenyl)-1-oxoethyl]-9(10*H*)-anthracenone (3v). To a solution of anthralin[50] (**1**; 1.00 g, 4.42 mmol), *p*-tolylacetic acid (**2v**, X = OH; 0.80 g, 5.30 mmol), and DCC (1.10 g, 5.30 mmol) in absolute THF (30 mL) was added dry pyridine (2.0 mL) under N_2. The reaction mixture was stirred at room temperature for 4 h, filtered, and the filtrate was evaporated. The residue was purified by chromatography and recrystallized from benzene/hexane (7/3) to afford **3v** as yellow needles (Table 3): FTIR 1711 (COOH), 1630 cm^{-1} (CO···HO); ^{1}H NMR ($CDCl_3$) δ 12.26 (2H), 7.54−6.59 (m, 10H), 5.31 (s, 1H), 3.33 (s, 2H), 2.26 (s, 3H). Anal. ($C_{23}H_{18}O_4$) C, H.

Method C. 4-[2-(4,5-Dihydroxy-10-oxo-9,10-dihydroanthracen-9-yl)-2-oxoethyl]phenylacetic Acid (3n). To a solution of anthralin[50] (**1**; 4.85 g, 21.5 mmol), 1,4-phenylenediacetic acid (**2n**, X = OH; 4.17 g, 21.5 mmol), and EDC (8.24 g, 43 mmol) in absolute THF (200 mL) was added dry pyridine (3.0 mL) under N_2. The reaction mixture was stirred for 2 h at 50 °C and an additional 12 h at room temperature. Then it was treated with 2 M HCl (200 mL), and the mixture was extracted with EtOAc (3 × 100 mL). The combined organic phase was washed with water, dried over Na_2SO_4, and the solvent was evaporated. The residue was purified by chromatography and recrystallized from benzene/THF (8/2) to afford a pale yellow powder (Table 3): FTIR 1736 (COOH), 1707

(CO), 1628 cm^{-1} (CO···HO); ^1H NMR (DMSO-d_6) δ 12.50−11.50 (br, 1H), 11.93 (s, 2H), 7.64−6.90 (m, 10H), 5.75 (s, 1H), 3.96 (s, 2H), 3.51 (s, 2H); MS *m/z* 402 (2.5), 226 (100). Anal. ($C_{24}H_{18}O_6$) C, H.

3a,**e**,**s**,**t**,**w**,**gg** were prepared as described.[12]

***N*-Hydroxy-*N*-methyl-4-[2-(4,5-dihydroxy-10-oxo-9,10-dihydroanthracen-9-yl)-2-oxoethyl]phenylacetamide (3o).** A solution of **3n** (1.00 g, 2.49 mmol), *N*-methyl-*N*-hydroxylamine (0.42 g, 4.97 mmol), and EDC (0.95 g, 4.97 mmol) in absolute DMF (20 mL) was stirred at room temperature for 5 h under N_2. Then cold 2 M HCl (100 mL) was added, the precipitate was filtered by suction, washed with water (100 mL), and dried under vacuum. The residue was treated with THF (100 mL), dried over Na_2SO_4, and the solvent was evaporated. The residues were purified by chromatography to give a yellow-brown powder (Table 3): FTIR 3417 (OH), 1713 (CO), 1630 cm^{-1} (CO···HO); ^1H NMR (DMSO-d_6) δ 11.93 (s, 2H), 9.96 (s, 1H), 7.65−6.88 (m, 10H), 5.75 (s, 1H), 3.95 (s, 2H), 3.64 (s, 2H), 3.09 (s, 3H); LSIMS (3-nitrobenzylacohol/CH_2Cl_2) *m/z* 431.4 (M^+). Anal. ($C_{25}H_{21}NO_6$) C, H.

Biological Assay Methods. Deoxyribose degradation as a measure of hydroxyl-radical generation,[12] HaCaT keratinocyte proliferation assay,[18] LDH release as a measure of membrane damage,[19] and inhibition of LTB_4 biosynthesis in bovine polymorphonuclear leukocytes[12] were described previously in full detail.

Cornified Envelope Assay. The cornified envelope is a well-recognized marker of terminal differentiation in keratinocytes.[38] The assay measures insoluble cross-linked protein envelopes based on previous reports.[39,51] HaCaT cells were cultured as described.[18] The medium of 144-h post-confluent cultures was replaced, and the test compounds were added from stock solutions (1, 5, 10 μM). These were prepared in DMSO and then diluted with Dulbecco's modified Eagle's medium; the final concentration of DMSO was 0.2% in the culture medium. Controls were performed with DMSO or medium alone. 48 h 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, 0.2% EDTA in PBS for 20 min at 37 °C. The detached cells from each well were treated with 10% sodium dodecyl sulfate and 2% β-mercaptoethanol (SDS/βME) in water (100 μL) with vigorous agitation. A sample (250 mL) of the suspension was applied to a presoaked, regenerated cellulose sheet (RC 60, Schleicher and Schuell, Dassel, Germany) over a dot-blot apparatus (Bio-Dot, BIO-RAD, Munich, Germany) using a presoaked, protein-free cellulose sheet (GB 002, Schleicher and Schuell) as a backing. The suspension was drained by gentle suction from below, and wells were washed with SDS/βME (3 × 400 μL). The RC 60 sheet was dried and then removed from the apparatus, submerged in ice-cold 7.5% TCA solution (500 mL), heated to 80 °C for 30 min, washed with ether/EtOH (1/1) for 10 min and then with ether (250 mL) for 10 min. The RC 60 sheet was dried and stained with a solution of 1% Coomassie blue G-250 in 7% acetic acid (500 mL) at 50 °C for 15 min. Then it was washed with 7% acetic acid at 50 °C for 5 min. This was repeated until the background was white (usually two times). The sheet was then transferred to a scanner (Hewlett-Packard Scan Jet 4c), and image analysis was performed with Optimas 6.1 (Media Cybernetics, Göttingen, Germany) on a Pentium Vectra computer. Indicated values are the differences of the amount of cross-linked protein in the presence of test compounds and vehicle control. Protein in envelope preparations was determined by the method of Bradford.[52]

Supporting Information Available: Analytical data for compounds **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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