Antipsoriatic Anthrones with Modulated Redox Properties. 3. 10-Thio-Substituted 1,8-Dihydroxy-9(10*H*)-anthracenones as Inhibitors of Keratinocyte Growth, 5-Lipoxygenase, and the Formation of 12(*S*)-HETE in Mouse Epidermis[1]

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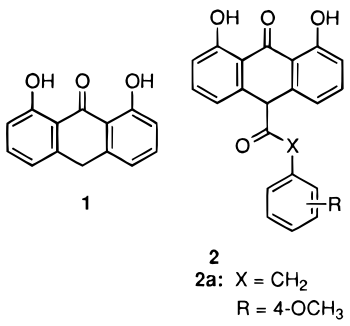
The synthesis of a series of 1,8-dihydroxy-9(10*H*)-anthracenones bearing sulfur-linked substituents in the 10-position is described. These compounds were evaluated for their ability to inhibit the growth of the human keratinocyte cell line HaCaT and the 5- and 12-lipoxygenase enzymes in bovine polymorphonuclear leukocytes and mouse epidermal homogenate, respectively. In addition, the following redox properties of the compounds were determined: reactivity against 2,2-diphenyl-1-picrylhydrazyl, generation of hydroxyl radicals as measured by deoxyribose degradation, and inhibition of lipid peroxidation in model membranes. Compounds **4e** and **4h** of this series compare favorably in the cellular assays with the antipsoriatic anthralin. They have the combined inhibitory action against leukotriene B_4 and 12(*S*)-HETE formation and are highly potent antiproliferative agents against keratinocyte growth. In contrast to anthralin, **4h**, 1,8-dihydroxy-10-[(4-hydroxyphenyl)thio]-9(10*H*)-anthracenone, is not cytotoxic as documented by the LDH activity released from cytoplasm of keratinocytes and does not enhance lipid peroxidation in model membranes.

Anthralin (**1**) is among the most widely used drugs in the treatment of psoriasis.[2] However, its clinical efficacy is limited by irritation of the nonaffected skin surrounding the psoriatic lesion and its staining of skin and clothing. These are significant drawbacks which are serious enough to prevent its use or reduce patient compliance.[3] Accordingly, the development of a topically effective antipsoriatic agent which should obviate the undesired side effects is highly desirable.[4,5] The mechanism of anthralin-induced skin irritation is not completely understood, but several studies have suggested that it may be associated in part with the formation of active oxygen species[6,7] or anthralin radicals[8,9] and subsequent lipid peroxidation.[7,10] Our strategy for overcoming these problems was to modulate the radical-generating intensity of anthralin and stabilize the 1,8-dihydroxy-9(10*H*)-anthracenone pharmacophore by modifying the critical 10-position of the molecule.

In a previous paper, we described the synthesis and structure−activity relationships for 10-acyl derivatives of anthralin.[11] This modification of anthralin, which gave rise to compounds of the general formula **2**, enhanced the activity against 5-lipoxygenase (LO) by two orders of magnitude. The compounds of this series appear to inhibit 5-LO by specific enzyme interaction rather than nonspecific redox inhibition. Currently, clinical trials in psoriasis are underway with **2a**.

In order to provide further insight into the anthracenone pharmacophore and the involvement of free radicals, we examined the effects of introducing electron-donating 10-thio substituents to see whether replacement of the electron-withdrawing carbonyl of the earlier series can provide analogs with both potent LO enzyme inhibitory and antiproliferative activities. This paper describes the design and synthesis of anthracenones that incorporate in their structure a potential antioxidant component (thioether side chain) and the results of relevant biological studies.

Chart 1



Chemistry

Application of the Captodative Effect. For the 1,8-dihydroxy-9(10*H*)-anthracenone-10-yl radical, two mesomeric forms **1**α,*β* (Scheme 1) account for the conjugative stabilization effect by the 9-keto function, an acceptor group at a vinylogous position. It has been postulated that the stability of free radicals is enhanced by the combined action of an electron-withdrawing and an electron-donating group attached to the same or to two vinylogous carbon atoms.[12] Accordingly, a donor substituent by its nature at the 10-position of the anthracenone nucleus contributes a stronger stabilization to the electrophilic radical **1**α,*β* than an acceptor substituent, a type of resonance stabilization called the captodative effect or push-pull substitution.[12,13] On the basis of this concept, a 10-thio group was assumed a suitable substituent to fulfill these requirements. In

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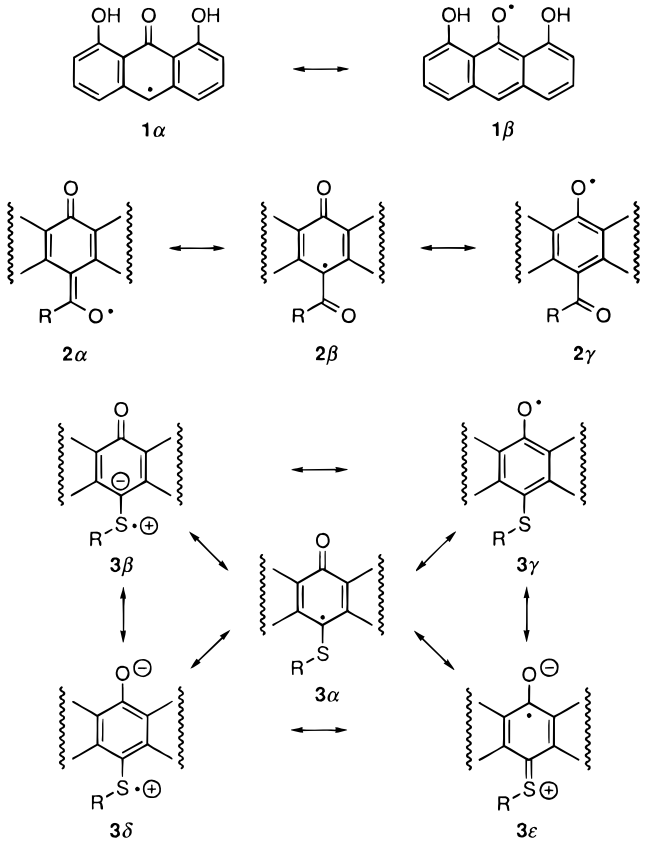
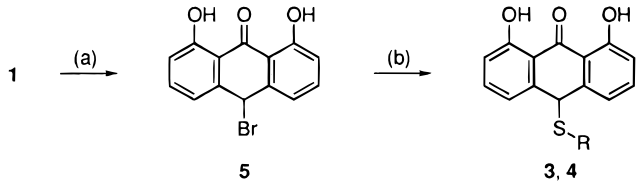
Scheme 1. Mesomeric Forms of Anthralin Radical **1**α,*β* and Stabilization by an Electron-Withdrawing Keto Substituent (**2**α−*γ*) and an Electron-Donating Thio Substituent (**3**α−*ϵ*)



this case, two substituents of opposite polarity act on the stabilization of the corresponding captodative radical and allow the drawing of five resonance structures **3**α−*ϵ*. By contrast, pairs of identical substituents act on the radical of 10-acyl derivative **2** and only three mesomeric forms **2**α−*γ* are possible. The stronger stabilization of **3** is further supported by an ESR investigation of electronic and conformational effects in similarly substituted phenoxyl radicals.[14] Compared to the parent phenol, the rotational barrier of a 4-methylthio-substituted phenoxyl radical was eight-fold increased, whereas no significant change was observed with a 4-acetyl group.[14] This shows that the CH_3S-substituent interacts strongly with the unpaired electron and effectively withdraws the spin density from the ring, which may not be substantial for the acetyl substituent.[14] Moreover, our preliminary ESR studies revealed that the reduction power of anthracenones substituted with an electron-withdrawing group in the C-10 position is impeded, whereas that of 10-thio derivatives is increased.[15] Selected anthracenones could be ranged according to the rate of reduction of a nitroxide radical into the following order:[15] **3j** > **1** > **2a**.

Synthesis. The 10-thio-substituted 1,8-dihydroxy-9(10*H*)-anthracenones (**3**, **4**) were synthesized readily according to Scheme 2. Thus, treatment of **1** with bromine provided 10-bromo-1,8-dihydroxy-9(10*H*)-anthracenone (**5**).[16] In contrast to earlier reports,[17,18] the desired compounds were obtained directly from the intermediate **5** by nucleophilic substitution at C-10 with 1.5−2 equivalents of appropriate mercaptans in the presence of catalytic amounts of trifluoroacetic acid,

Scheme 2[a]



[a] R is defined in Table 1. Reagents: (a) Br_2, CS_2, 50 °C; (b) RSH, TFA, CH_2Cl_2, room temperature.

which strongly reduced the reaction time as compared to the noncatalyzed reaction. Compounds containing an arylthio or aralkylthio substituent (**4**) at C-10 were obtained according to the same procedure.

Biological Evaluation

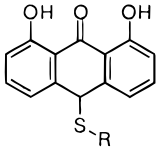
Psoriasis is a widespread, inflammatory and scaling skin disease, which is characterized by increased cell proliferation of the epidermis.[19] Both features of psoriasis, hyperproliferation and inflammation, are resolved following topical therapy with anthralin. Although there is no appropriate animal model of psoriasis, the biological activity of drugs useful as antipsoriatic agents may be evaluated by their antiproliferative activity in cell cultures,[20] which may be critical in resolving hyperproliferation of psoriasis. Furthermore, inhibition of the biosynthesis of both leukotrienes (LT) and hydroxyeicosatetraenoic acids (HETE) may be appropriate to manage the inflammatory component of the disease.[21] On the basis of this information, we evaluated the ability of the novel compounds to inhibit the growth of human keratinocytes[1] and the 5- and 12-LO pathways in bovine polymorphonuclear leukocytes[11] (PMNL) and epidermal homogenate of mice,[22] respectively.

Antiproliferative Activity. *In vitro* cultured cell systems are useful tools in identifying new topical antipsoriatic agents. HaCaT keratinocytes can be used as a model for highly proliferative epidermis, e.g. psoriasis, and this nontransformed human cell line was described as an extremely sensitive target for the antiproliferative action of anthralin.[23] Proliferation of the keratinocytes was determined directly by counting the dispersed cells under a phase-contrast microscope after 48 h of treatment.

The compounds in Table 1 were tested for antiproliferative effects as demonstrated by reduction in cell number over time as compared to control plates. Except for compounds **3a** and **4l**, all of them exhibited activity against cell growth in the micromolar range. Although there were no obvious requirements for potent antiproliferative activity, the (2-amino- (**4e**) and (4-hydroxyphenyl)thio (**4h**) compounds were highly potent inhibitors with IC_{50} values as low as 0.1 μM. These two compounds were about six-fold more potent than anthralin, which had an IC_{50} of 0.6 μM in this assay. However, only minor structural changes, such as variation of the position of the amino or hydroxy function at the phenyl ring (**4f**,**g**) or methylation (**4d**), strongly decreased potency.

Lactate Dehydrogenase Release. A major concern in the testing of potential inhibitors of cell growth is to confirm that the drug does not interfere with the functioning of cell membrane by causing leakage of cytoplasm through it. Anthralin has been reported to

Table 1. Deoxyribose Degradation, 5-LO Inhibition in Bovine PMNL, 12-LO Inhibition in Mouse Epidermis, Antiproliferative Activity, and Cytotoxicity against HaCaT Cells by 10-Thio-Substituted 1,8-Dihydroxy-9(10*H*)-anthracenones



compd	R	DD (•OH)[a]	5-LO IC_{50} (μM)[b]	12-LO IC_{50} (μM)[c]	AA IC_{50} (μM)[d]	LDH (mU)[e]
3a	CH_2CO_2H	6.12 ± 1.02	>30	30(84)	>5	ND
3b	$CH_2CO_2CH_3$	4.89 ± 1.01	13	>30	1.9	ND
3c	$CH_2CO_2C_2H_5$	4.51 ± 0.84	>30	>30	4.5	ND
3d	$CH_2CO_2C_4H_9$	4.19 ± 0.39	>30	30(66)	2.1	ND
3e	$CH(CH_3)COOH$	9.34 ± 0.33	>30	15	0.2	ND
3f	$CH_2CH_2CO_2H$	6.34 ± 0.26	2	30(74)	1.9	ND
3g	$CH_2CH_2CO_2CH_3$	5.25 ± 0.59	15	27	1.1	133[f]
3h	$CH_2CH_2CO_2C_2H_5$	5.52 ± 0.27	>30	30(69)	1.6	124[f]
3i	$CH_2CH_2CO_2C_4H_9$	5.38 ± 0.75	30(62)	30(62)	2.3	ND
3j	$CH_2CH(NH_2)COOH$	2.33 ± 0.13	>30	30(71)	>5	ND
3k	CH_2CH_3	4.23 ± 0.15	>30	>30	1.4	ND
3l	CH_2CH_2OH	2.49 ± 0.25	2	>30	1.2	136[f]
3m	$CH_2CH_2CH_3$	2.81 ± 0.78	>30	>30	1.2	117[f]
3n	$CH_2CHOHCH_2OH$	2.45 ± 0.16	9	>30	1.7	ND
3o	$CH_2CH_2CH_2SH$	4.09 ± 0.06	>30	30(58)	0.8	369
4a	C_6H_5	4.13 ± 0.41	8	30(55)	3.7	ND
4b	$2\text{-}CH_3OC_6H_4$	4.33 ± 1.02	6	3	4.2	ND
4c	$3\text{-}CH_3OC_6H_4$	4.29 ± 0.07	15	30(62)	1.8	ND
4d	$4\text{-}CH_3OC_6H_4$	1.59 ± 0.16	3	30(69)	1.7	137[f]
4e	$2\text{-}H_2NC_6H_4$	4.76 ± 0.56	2	6	0.1	255
4f	$4\text{-}H_2NC_6H_4$	1.49 ± 0.32	7	>30	0.9	184
4g	$2\text{-}HOC_6H_4$	3.70 ± 0.11	5	30(73)	1.7	174
4h	$4\text{-}HOC_6H_4$	3.61 ± 0.10	2	3	0.1	148[f]
4i	$CH_2C_6H_5$	3.74 ± 0.36	>30	30(54)	1.4	ND
4j	$CH_2C_6H_4OCH_3$	5.01 ± 0.02	>30	30(77)	3.0	ND
4k	$CH_2CH_2C_6H_5$	2.78 ± 0.52	30(58)	>30	4.7	ND
4l	$CH_2CH_2CH_2C_6H_5$	2.71 ± 0.10	30	>30	>5	ND
anthralin (**1**)		2.89 ± 0.14	37	9	0.6	294

[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] Inhibition of 5-HETE and LTB_4 biosynthesis in bovine PMNL. [c] Inhibition of 12(*S*)-HETE biosynthesis in mouse epidermal homogenates. Inhibition was significantly different with respect to that of the control; $N = 3$ or more, $P < 0.01$. Values in parentheses are percent inhibition at the indicated concentrations (μM), and standard errors average 10% of the indicated values. Nordihydroguaiaretic acid (NDGA) was used as the standard inhibitor for 5- and 12-LO ($IC_{50} = 0.4$ and 21 μM, respectively). [d] Antiproliferative activity against HaCaT cells. Inhibition of cell growth was significantly different with respect to that of the control, $N = 3$, $P < 0.01$. [e] Activity of LDH (mU) release in HaCaT cells after treatment with 2 μM test compound ($N = 3$, SD < 10%). [f] Values are not significantly different with respect to vehicle control. ND = not determined.

cause membrane damage, but did not directly lead to substantial membrane destruction.[23] Cytotoxicity against the cell cultures by the potent cell growth inhibitors was assessed by the activity of lactate dehydrogenase (LDH) activity released into the culture medium. The release of LDH is commonly used as indicator of plasma membrane damage. In this assay, LDH release by anthralin significantly exceeded that of the vehicle control. On the other hand, the activity of the novel compounds was due to cytostatic rather than cytotoxic effects, as LDH release was unchanged as compared to controls. Except for thiol **3o**, which shows increased membrane damage as compared to anthralin, in all other cases cytotoxic effects were either strongly reduced (**4e**−**g**) or not apparent at 2 μM (**3g**,**h**,**l**,**m**, **4d**,**h**) compared to control cultures.

Inhibition of 5-Lipoxygenase. Formation and release of arachidonic acid metabolites from PMNL[24] and keratinocytes[25] participate in amplifying the inflammatory response. Among the most active of these metabolites is LTB_4, generated by the action of 5-LO. Accordingly, potential antipsoriatic drugs are evaluated for their ability to inhibit the production of LTB_4.[26−29] Considering that the biosynthesis of LTB_4 is a radical-based oxidation,[30] many LO inhibitors can be categorized as antioxidants/free radical scavengers.[31−35]

Anthralin itself is only a moderate 5-LO inhibitor. In isolated bovine PMNL it inhibited the production of LTB_4 and 5-HETE with an IC_{50} of 37 μM.[11] As shown in Table 1, we found that many compounds of the new 10-thio-substituted series were far more potent than anthralin. We previously reported substantial increases in 5-LO inhibitory activity by the introduction of terminal phenyl rings on the 10-substituent of the anthracenone.[11] A similar approach within the 10-thio series resulted also in active compounds, even though their inhibitory activity against the 5-LO enzyme and the beneficial effect of the terminal phenyl ring was less pronounced as that observed with 10-phenylacyl derivatives.[11] Phenylthio-substituted analogs with the phenyl ring directly attached to the sulfur linkage (**4a**−**h**) all demonstrated reasonable activity, whereas insertion of methylene groups (**4i**−**l**) resulted in loss of 5-LO inhibition. Of the alkylthio-substituted compounds, only the thiopropanoic acid **3f** and thioethanol **3l** exhibited potency comparable to that of **4e** or **4h**. In contrast to previous work,[1,33] no correlation between 5-LO inhibition and lipophilicity of the compounds (log *P* values are given in Table 3) was observed.

Inhibition of 12(*S*)-HETE Formation. Although the literature of 5-LO inhibitors is vast,[30] not many compounds have been tested for 12-LO inhibitory activity. However, 12-HETE is the major LO product found in the skin[36] and has been demonstrated to be present in high levels in psoriatic epidermis.[37] Furthermore, the characterization of LO activity in human epidermis indicates that germinal layer keratinocytes contain a highly active 12-LO which is selectively expressed at a higher level during psoriatic inflammation.[38] 12-HETE stimulates keratinocyte proliferation and induces histological changes characteristic of psoriasis.[39−41] Studies on the evaluation of 12-LO inhibitors have utilized platelets as the source of the enzyme in most cases.[42,43] Observations that potent inhibitors of the epidermal 12-LO did not inhibit platelet derived 12-LO suggest the existence of two distinct enzymes.[44,45] Anthralin showed higher selectivity for epidermal 12-LO *versus* the platelet enzyme (IC_{50} values are 9 and 30 μM, respectively).[22] With psoriasis as target for therapy, potential 12-LO inhibitors have to be evaluated using epidermal 12-LO.[21]

In this study we used epidermal strips from mouse skin to investigate the effects of the novel 10-thio-substituted analogs. To exclude oxidation of arachidonic acid by a nonspecific free radical mechanism which affords racemic 12-HETE, the absolute stereochemistry of 12-HETE was determined by chiral phase HPLC to confirm the presence of the *S*-enantiomer.[22]

Introduction of a 10-thio side chain containing polar or nonpolar groups resulted in decrease in potency as compared to anthralin, with the exception of some phenylthio-substituted analogs. As can be seen in Table 1, (2-methoxy- (**4b**) and (4-hydroxyphenyl)thio substitution (**4h**) at C-10 resulted in a three-fold increase in inhibition of 12(*S*)-HETE formation as compared to anthralin, and also **4e** is more active than the antipsoriatic drug. Although there was no correlation between 5- and 12-LO inhibition, compound **4h** was an equipotent inhibitor of both the LO enzymes.

Antioxidant and Prooxidant Determination. There is no dispute that infiltration of PMNL of the epidermis is a characteristic feature of the established psoriatic lesion.[19] Several lines of evidence suggest that the release of oxidants by PMNL and macrophages leads to injury to the tissue, and a similar role of active oxygen species has been identified in psoriasis.[46,47] The activity of xanthine oxidase, an enzyme capable of generating superoxide radical, is increased in psoriatic epidermis.[48] Uncontrolled production of active oxygen species leads to peroxidative damage to membranes of the skin, a tissue that is particularly vulnerable to the effects of these species.[49] The increased exposure of the skin to prooxidants together with an insufficient capacity of the antioxidative system to respond seems to play an important role in the induction of psoriasis by exogenous factors.[50] To counteract this oxidative stress, compounds that incorporate in their structure a potential antioxidant component and interact with free radicals are of interest. Therefore, we determined the reactivities of the compounds with the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH).[11] In general, the novel anthracenones were far more reactive (k_{DPPH} > 100 M^{-1} s^{-1}, data not shown) than anthralin (24 ± 4.2 M^{-1} s^{-1}),[11] reflecting the reducing capability of the 10-thio substituent.

Table 2. Half-lives ($t_{1/2}$, h) of Selected 10-Thio-Substituted 1,8-Dihydroxy-9(10*H*)anthracenones in DMSO and Ethanol at 25 °C and Acidity Constants (pK_a) in Aqueous Solution

compd	$t_{1/2}$ (h) DMSO	$t_{1/2}$ (h) ethanol	pK_a
3i	11	2	5.7
4a	28	12	5.4
4d	32	21	7.9
4e	26	20	8.4
4h	55	34	6.2
4j	23	5	6.1
4k	4	3	ND
anthralin (**1**)	18[57]	3[57]	9.5[55]

Prooxidant properties of the compounds were defined by the deoxyribose assay.[11] The release of malondialdehyde (MDA) is indicative of hydroxyl-radical formation. As already observed for catechol or pyrogallol compounds of our earlier series,[1,11] the potential antioxidant thio component once more increased the release of MDA (Table 1), indicating enhanced formation of hydroxyl radicals; compounds **3j**,**l**,**n** and **4d**,**f** were exceptions. This is a further documentation that certain substances can have prooxidant effects under some reaction conditions and cannot be classified simply as antioxidants on the basis of experiments performed with lipid systems.[51,52] Although this is somewhat disappointing, however, compounds that enhance oxidative damage to deoxyribose are not necessarily prooxidants with respect to DNA bases.[53]

Free radical intermediates and end-products derived from lipid peroxidation are important components in inflammatory diseases.[54] Thus, agents that inhibit initiation or propagation of lipid peroxidation may be helpful in preventing tissue injury. Representative compounds were evaluated for their efficacy to block 2,2′-azobis(2-amidinopropane) hydrochloride (AAPH) induced lipid peroxidation in bovine brain phospholipid liposomes[11] and gave IC_{50} values of 24 (**3c**, **4a**,**h**), 46 (**3n**), 62 (**4i**,**k**), and for **4l** 85 μM (data not shown). By contrast, anthralin itself was not effective against AAPH-induced lipid peroxidation but rather enhanced lipid peroxidation both *in vivo* and in model membranes.[7,10] Appropriate control experiments revealed that this was not the case with the thio analogs, despite their ability to generate hydroxyl radicals.

Stability. Anthralin staining is an important limitation of its therapeutic use and is primarily due to its oxidation to the anthralin radical which dimerizes or polymerizes to give inactive products such as bianthrone and the so-called anthralin-brown, respectively.[2] With the 10-thioether side chain, this side effect should be reduced because of steric hindrance to radical dimerization. To prove this hypothesis, we have determined the decomposition of anthralin and some of its 10-thio derivatives in dimethyl sulfoxide (DMSO) and ethanol by RP-HPLC over a 10-day period at 25 °C. Table 2 shows the half-lives of selected compounds in the corresponding solvents. In general, alkylthio substitution (**3i**, **4k**) decreased the stability as compared to anthralin, whereas phenylthio-substituted compounds (**4a**,**d**,**e**,**h**) were considerably more stable. The decomposition of the compounds was more favored in the protic solvent ethanol than in the aprotic DMSO.

Furthermore, the acidity of the compounds may have a considerable impact on their stability, because an-

thralin anion is the key intermediate in the oxidation process, which involves one-electron transfer from the anion to oxygen to give anthralin-10-yl radical and superoxide anion.[10] Thus, absorbance spectroscopy was employed to obtain the pK_a values of the 10-substituted derivatives of anthralin. However, there was no relationship found between stability and acidity of the compounds. The 10-thio derivatives were all more acidic than anthralin which had a pK_a value of 9.5.[55] The decomposition products of the 10-thio derivatives in the organic solvents detected by HPLC were the stable 1,8-dihydroxy-9,10-anthracenedione, which does not polymerize, and the corresponding thiols, whereas the dimer bianthrone and anthralin itself made no contribution to the overall degradation. Thus, a phenylthioether side chain at C-10 of anthralin greatly enhances its stability in both protic and aprotic solvents and also prevents the anthracenone molecule from being polymerized to anthralin-brown.

The better *in vitro* activity in some assays observed with compounds **4** having the phenyl ring directly attached to the sulfur linkage compared to those with methylene spacers may thus be directly related to the improved stability also seen with those compounds.

Summary and Conclusions

10-Thio-substituted anthracenones were synthesized and evaluated in various assays. Proper selection of the 10-thiophenyl substituent seemes to be critical for potent *in vitro* activity, since this is strongly dependent of the nature and position of the substituent on the phenyl ring. 2-Aminophenyl and 4-hydroxyphenyl groups are found to be optimal. Overall, compound **4h** of this series has the best biological profile both with respect to inflammatory and hyperproliferative features of the disease state and compares favorably in the cellular assays with the antipsoriatic drug anthralin. The amount of hydroxyl-radical formation by **4h** is comparable to that of anthralin, but contrary to this drug,[7,10] **4h** does not enhance lipid peroxidation in model membranes and is not cytotoxic as documented by the LDH activity released from cytoplasm. It is one of the most potent inhibitors of 12(*S*)-HETE formation in mouse epidermis of the anthracenone class obtained to date and also inhibits leukotriene biosynthesis in intact PMNL. Furthermore, this compound is highly potent and even more efficient as an inhibitor of human keratinocytes growth and also more stable than anthralin.

In conclusion, this study presents novel dual-function inhibitors of both the 5- and 12-LO pathway and the growth of human keratinocytes and suggests that nontoxic agents of this type may be useful for the treatment of psoriasis.

Experimental Section

Melting points were determined with a Büchi 510 melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded with a Bruker Spectrospin WM 250 spectrometer (250 MHz), using tetramethylsilane as an internal standard. Fourier-transform IR spectra (KBr) were recorded on a Nicolet 510M FTIR spectrometer. UV spectra were recorded on a Kontron 810 spectrometer. Mass spectra 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 analyzed with the software Peaks on an Apple Macintosh computer.

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

compd	log *P*	formula[a]	mp, °C	yield, %	anal.[b]
3a	2.35	$C_{16}H_{12}O_{5S}$	151−152[c]	46	C, H
3b	3.53	$C_{17}H_{14}O_5S$	130−131[d]	48	C, H
3c	4.45	$C_{18}H_{16}O_5S$	92−93	85	C, H
3d	4.41	$C_{20}H_{20}O_5S$	90−91	66	C, H
3e	2.77	$C_{17}H_{14}O_5S$	168−169	37	C, H
3f	3.00	$C_{17}H_{14}O_5S$	147−148[e]	65	C,[f] H
3g	3.69	$C_{18}H_{16}O_5S$	88−89[g]	75	C, H
3h	4.59	$C_{19}H_{18}O_5S$	86−87	54	C, H
3i	4.55	$C_{21}H_{22}O_5S$	72−73	75	C, H
3j	2.35	$C_{17}H_{15}NO_5S$	176−178[h]	44	C, H
3k	2.97	$C_{16}H_{14}O_3S$	117−118[i]	63	C, H
3l	3.16	$C_{16}H_{14}O_4S$	146−147[j]	91	C, H
3m	4.57	$C_{17}H_{16}O_3S$	84−85	83	C, H
3n	2.73	$C_{17}H_{16}O_5S$	152−153[k]	75	C, H
3o	4.33	$C_{17}H_{16}O_3S_2$	58−59	45	C, H
4a	4.54	$C_{20}H_{14}O_3S$	149−150[l]	68	C,[m] H
4b	4.42	$C_{21}H_{16}O_4S$	135	65	C, H
4c	4.50	$C_{21}H_{16}O_4S$	96−97	72	C, H
4d	4.51	$C_{21}H_{16}O_4S$	143−144	77	C, H
4e	4.05	$C_{20}H_{15}NO_3S$	132−133	45	C, H, N
4f	3.65	$C_{20}H_{15}NO_3S$	160−161	41	C, H, N
4g	3.92	$C_{20}H_{14}O_4S$	151−152	52	C, H
4h	3.69	$C_{20}H_{14}O_4S$	189−190	46	C, H
4i	4.87	$C_{21}H_{16}O_3S$	155[n]	85	C, H
4j	4.83	$C_{22}H_{18}O_4S$	105−106	58	C, H
4k	4.97	$C_{22}H_{18}O_3S$	115−116	75	C, H
4l	4.78	$C_{23}H_{20}O_3S$	72−73	80	C, H

[a] All new compounds displayed ^{1}H NMR, FTIR, UV, and MS spectra consistent with the assigned structure. [b] Elemental analyses were within ±0.4% of calculated values. [c] Literature[58] mp 151−152 °C. [d] Literature[58] mp 120−122 °C. [e] Literature[58] mp 152−153 °C. [f] C: calcd, 61.82; found, 61.07. [g] Literature[58] mp 92−94 °C. [h] Literature[18] mp 176−180 °C. [i] Literature[58] mp 117−118 °C. [j] Literature[58] mp 146−147 °C. [k] Literature[58] mp 154−155 °C. [l] Literature[18] mp 145 °C. [m] C: calcd, 71.84; found, 71.30. [n] Literature[18] mp 153 °C.

General Procedure for the Synthesis of 10-(Alkylthio)- and 10-[(*ω*-Substituted-Phenyl)thio]-1,8-dihydroxy-9(10*H*)-anthracenones. 1,8-Dihydroxy-10-[(4-hydroxyphenyl)thio]-9(10*H*)-anthracenone (4h). To a solution of 10-bromo-1,8-dihydroxy-9(10*H*)-anthracenone[16] (**5**, 305 mg, 1 mmol) and 0.1 mL of trifluoroacetic acid in dry CH_2Cl_2 (20 mL) was added dropwise a solution of 4-mercaptophenol (252 mg, 2 mmol) in dry CH_2Cl_2 (10 mL) under N_2. The reaction mixture was stirred at room temperature for 6 h. The solvent was removed and the residue purified by column chromatography on silica gel (E. Merck, 70−230 mesh) to provide yellow crystals (Table 3): ^{1}H NMR (250 MHz, $CDCl_3$) δ 11.83 (s, 2H), 7.49 (t, 2H, J = 8.0 Hz), 7.01 (dd, 2H, J = 7.5 Hz), 6.87 (dd, 2H, J = 8.4 Hz), 6.55 (s, 4H), 5.35 (s, 1H), 4.91 (s, 1H); FTIR 3436 (OH), 1630 cm^{-1} (CO); MS m/z 350 (11), 225 (100). Anal. $C_{20}H_{14}O_4S$ (C, H).

log *P* Determination. A standard reversed-phase HPLC procedure was used. MeOH/water/HOAc (77/23/0.1), adjusted to pH 5.5 with concentrated NH_3, was used as eluant. Calibration was performed as described.[11] log P values as a measure of lipophilicity are given in Table 3.

Determination of the Reducing Activity against 2,2-Diphenyl-1-picrylhydrazyl.[11] 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.[11] 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 described.[11] 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.

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

Cell Culture and Determination of Cell Growth. HaCaT cells[56] were cultivated, and the cell proliferation assay was performed as previously described.[1] After 48 h of incubation, cell growth was determined by enumerating the dispersed cells by phase contrast microscopy. Inhibition was calculated by the comparison of the mean values of the test compound ($N = 3$) with the control ($N = 6-8$) activity: (1 − test compound/control) × 100. Inhibition was statistically significant compared to that of the control (Student's t-test; $P < 0.05$). Each IC_{50} value was derived by interpolation of a log inhibitor concentration versus response plot using four or more concentrations of the compound, spanning the 50% inhibition point.

Lactate Dehydrogenase Release.[23] HaCaT cells were incubated with the test compounds (2 μM) for 4 h at 37 °C. Extracellular LDH activity was measured using the UV method with pyruvate and NADH and is expressed in mU/mL. Appropriate controls with the vehicle were performed ($P < 0.01$; $N = 3$, SD < 10%).

Stability Studies.[57] The test compounds (1 mM) were dissolved in the pertinent solvents (p.a.) and were kept in the dark at room temperature. Aliquots of 20 μL were withdrawn after appropriate time and subjected to HPLC analysis. Eluant: methanol/water/acetic acid (77/23/0.1, adjusted to pH 5.5 with NH_3); flow rate 1 mL/min; 200 bar; detected at 254 nm.

Determination of the pK_a Values.[57] Absorption spectra of the compounds were obtained in buffer solutions ranging from pH 2.5−12.0. The pH values of the solutions were measured with a Knick type 647 digital pH meter and an Ag/AgCl electrode (Ingold) and corrected, if necessary. The absorbance at pH 2.5 served as the absorbance of the neutral species, whereas that at pH 12.0 as the absorbance of the completely deprotonated species. The pK_a values were determined from titration curves from absorbances at appropriate wavelengths.

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