Simple Analogues of Anthralin: Unusual Specificity of Structure and Antiproliferative Activity†

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Fifty-nine simple analogues of the antipsoriatic agent, anthralin, have been prepared by modifying the positions of the 1,8-hydroxyl groups, replacement of the hydroxyl groups, substitution at the oxygen functions, introduction of additional functional groups into various positions of the anthracenone nucleus, or removal of particular structural elements. The compounds were evaluated for their antiproliferative action against human keratinocytes and inhibition of the generation of leukotriene B_4 in polymorphonuclear leukocytes, which may be useful to resolve the proliferative and inflammatory aspects of psoriasis, respectively. Even though many anthracenones were more potent inhibitors of leukotriene biosynthesis than anthralin, none of the compounds was substantially more effective as this drug in suppressing keratinocyte cell growth. There is an absolute requirement for two hydroxyl groups *peri* to a hydrogen bond acceptor such as a keto or an imino group for high potency. In addition to further delineating the nature of the pharmacophore for this class of compounds, also naphthalenedione with a *peri* hydroxyl group was identified as a pharmacophore with antiproliferative activity against keratinocyte growth.

Anthralin (dithranol, **1**) was first developed in 1916 as a substitute for chrysarobin (**5f**; Chart 1). It is still today among the most frequently used antipsoriatic drugs. However, the benefits of the drug are limited by its undesirable proinflammatory effects on the skin or staining of the skin and clothing.[1] Thus patient compliance is reduced, and the development of derivatives which should obviate the undesired side effects is highly desirable.

Over a considerable period of time, several research groups have attempted to obtain therapeutic agents with less side effects based on anthralin. The primary structural changes introduced into the 9(10*H*)-anthracenone chemotype have been (i) repositioning of the hydroxyl groups, (ii) replacement of the hydroxyl groups by other substituents, (iii) esterification of the tautomeric 1,8,9-trihydroxyanthracene form, and (iv) substitution at the 10-position of the anthracenone nucleus. The latter two, acetylation of the oxygens or butyrylation of C-10 of anthralin, have led to triacetoxyanthracene[2] (**4f**) and butantrone[3] (**6k**), respectively. Although the staining effects associated with therapy appeared to be less pronounced than in the case of anthralin, inflammation of the nonaffected skin surrounding a psoriatic lesion still appears to be of clinical concern. Indeed, later reports have indicated that these compounds were less effective and more irritating than anthralin itself.[4,5]

The pioneering work of Unna,[6] in which several oxidation states of anthracenediones and anthracenones with relocated hydroxyl groups were tested on psoriatic patients, had shown that the antipsoriatic activity was lost if significant modifications were made to the structure. There have been further investigations of Krebs and Schaltegger on the structure specificity of this class of compounds.[7] From these studies, 1-hydroxy-9(10*H*)-anthracenone (**3b**) was suggested as the so-called "minimum structure for antipsoriatic activity", in which even minor structural changes resulted in inactive compounds.[7] Later it was shown that when one of the C-10 hydrogen atoms is replaced by an acyl group the antipsoriatic activity was preserved,[3] whereas substitution by alkyl groups gave inactive compounds.[8] However, clinical structure−activity studies on antipsoriatic anthracenones have not yet been quantified and subjected to statistical analysis.

Another problem that complicates structure−activity relationships is documented by the result of our recent study that gave cause to question the long-assigned structures of some anthralin analogues, i.e., 1,8-dimethoxy- (dimethylanthralin, **4h**) and 4,5-dihydroxy-9(10*H*)-anthracenone (isoanthralin, **2d**).[9] Likewise, it is not always clear whether the ascribed structures of many analogues tested are correct, since neither experimental data nor proof of structure were given for them.[6,10] In support of this, the product of the reduction of 1,2-dihydroxy-9,10-anthracenedione with aqueous ammonia and zinc dust was named anthrarobin and introduced into psoriasis therapy as a mild substitute for the skin-irritating chrysarobin.[11] The reported structure in the literature was 1,2-dihydroxy-9(10*H*)-anthracenone (**2a**).[11] However, later studies revealed that the major product of the reduction was the 3,4-dihydroxy-9(10*H*)-anthracenone (**2c**), and this compound had been evaluated for antipsoriatic activity.[12]

Furthermore, we have shown that the acylation of anthralin with acetylsalicylic acid chloride leads to the *O*-acylated product **4c**,[13] rather than the previously described 10-(acetylsalicyl)anthralin.[14] In view of this

† Dedicated to Prof. Dr. W. Wiegrebe, Regensburg, on the occasion of his 65th birthday.

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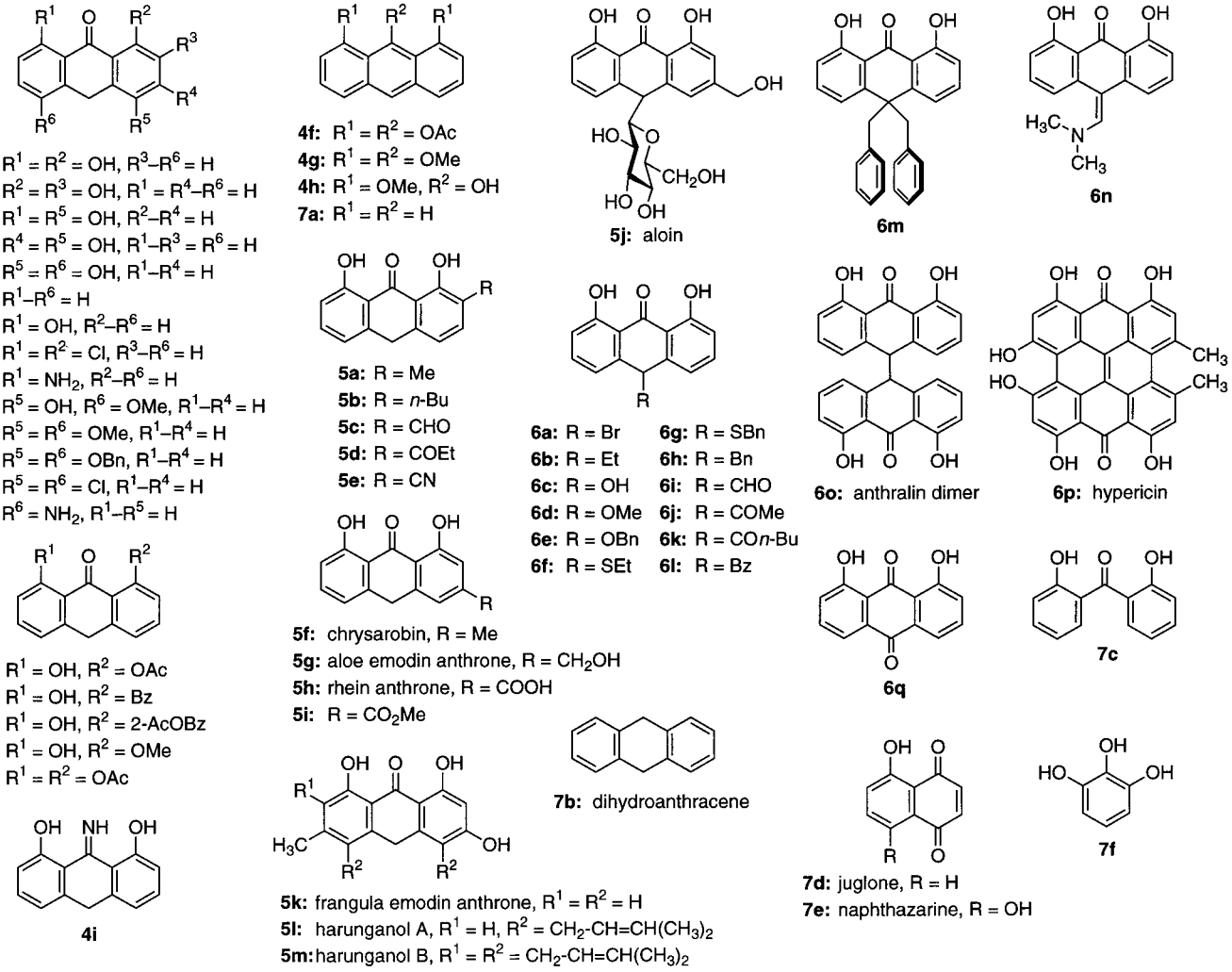
Chart 1



confusion some biological studies on anthralin derivatives have to be reconsidered. Therefore, a fundamental study on the structure specificity of anthralin was deemed necessary to understand the structure−activity relationships of the synthesized analogues and to facilitate further design.

In recent studies, we have engaged in substitution on the anthracenone structure to develop novel analogues of anthralin as potential antipsoriatic drugs. We synthesized different series of compounds that retained the hydroxyl groups in the appropriate 1,8-positions on the anthracenone nucleus.[15−18] Herein, we report on structure−activity relationships associated with changes of the position or nature of functional groups at the nucleus of anthralin.

Chemistry

The simple analogues of anthralin synthesized can be divided into five groups: (i) structures **2** had the 1,8-hydroxyl groups moved to different positions or (ii) substituted by other functional groups (**3**), (iii) the oxygen functions were substituted (**4**), (iv) additional functional groups were introduced into various positions of the anthralin nucleus (**5**, **6**), or (v) particular structural elements were removed (**7**).

Anthracenones **1**, **2a**−**d**, **3a**−**i**, and **5a**−**i** were prepared from the corresponding anthracenediones. The traditionally employed method that leads preferentially to the *peri*-substituted anthracenones uses stannous chloride in acetic acid−hydrochloric acid, whereas sodium dithionite in the presence of DMF in neutral solution selectively reduces the carbonyl group flanked by the *peri* substituents of the anthracenedione to afford the corresponding anthracenones with the substituents remote from the keto group (Scheme 1).[19]

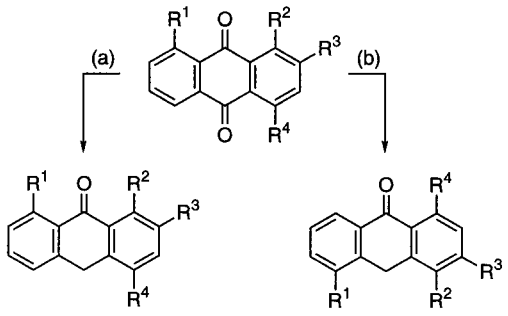
9(10*H*)-Anthracenimine **4i** (Chart 1) was obtained by treatment of **1** with aqueous ammonia in methanol at 0 °C. The 10-benzyloxy-substituted derivative **6e** (Chart 1) was obtained from 10-bromoanthralin[20] (**6a**) and benzyl alcohol in the presence of catalytical amounts of trifluoroacetic acid.[17] All other test compounds were known in the literature or were commercial products (Chart 1).

Noncommercially available anthracenedione precursors were prepared by established literature procedures, as indicated in the Experimental Section. Anthracenedione **8c** was prepared by Kornblum oxidation[21] of the benzyl bromide **8a** followed by ether cleavage of **8b** with HBr (Scheme 2). The aldehyde **8b** was also directly converted[22] into nitrile **8d** with hydroxylamine.

Biological Evaluation and Discussion

The biological testing procedures applied in this study were similar to those in our earlier work.[15,16] Evaluation of the usefulness of a potential antipsoriatic drug requires the satisfaction of two criteria. These include

Scheme 1[a]



1: $R^1 = R^2 = OH$, $R^3 = R^4 = H$ **2c:** $R^1 = R^4 = H$, $R^2 = R^3 = OH$

2a: $R^1 = R^4 = H$, $R^2 = R^3 = OH$ **2d:** $R^1 = R^2 = OH$, $R^3 = R^4 = H$

2b: $R^1 = R^4 = OH$, $R^2 = R^3 = H$ **3e:** $R^1 = OH$, $R^2 = OMe$, $R^3 = R^4 = H$

3b: $R^1 = OH$, R^2–$R^4 = H$ **3f:** $R^1 = R^2 = OMe$, $R^3 = R^4 = H$

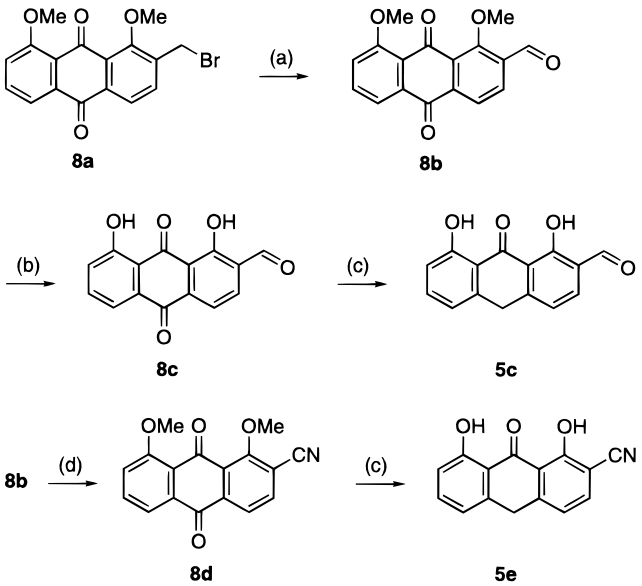
3c: $R^1 = R^2 = Cl$, $R^3 = R^4 = H$ **3g:** $R^1 = R^2 = OPh$, $R^3 = R^4 = H$

3d: $R^1 = NH_2$, R^2–$R^4 = H$ **3h:** $R^1 = R^2 = Cl$, $R^3 = R^4 = H$

3i: $R^1 = NH_2$, R^2–$R^4 = H$

[a] Reagents: (a) $SnCl_2$, HCl, HOAc, 118 °C; (b) $Na_2S_2O_4$, DMF, H_2O, 90 °C, N_2.

Scheme 2[a]



[a] Reagents: (a) DMSO, $NaHCO_3$, 40 °C; (b) 62% HBr, HOAc, 118 °C; (c) $SnCl_2$, HCl, HOAc, 118 °C; (d) $NH_2OH{\cdot}HCl$, HCOOH, 100 °C.

the demonstration that the compound is able to arrest both the excessive growth of keratinocytes and the inflammatory component of psoriasis.[23] Compounds that are targeted toward only one aspect of the disease are unlikely to be totally beneficial. Therefore, we determined the biological activity of the simple analogues of anthralin in terms of their antiproliferative action in human keratinocyte cell cultures, which may be critical in resolving hyperproliferation of psoriasis. Furthermore, evidence that polymorphonuclear leukocytes (PMNL) also play an important role in the pathogenesis of psoriasis and the recognition that the 5-lipoxygenase (5-LO) pathway is altered in psoriasis has prompted us to study the inhibition of the proinflammatory leukotriene B_4 (LTB_4) and 5-hydroxyeicosatetraenoic acid (5-HETE) in bovine PMNL by anthralin analogues.

As the mode of action and induction of side effects of anthralin are related to its redox activity leading to the production of oxygen radicals,[24,25] the anthralin analogues were further evaluated for their reactivity against the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), as a measure of their antioxidant potential, and their ability to generate hydroxyl radicals (prooxidant potential).

Inhibition of 5-Lipoxygenase. LTB_4 can effect keratinocyte proliferation,[26,27] and cultured HaCaT keratinocytes express the 5-LO gene.[28] Therefore, potential antipsoriatic drugs are evaluated for their ability to inhibit the production of LTB_4.[29−32]

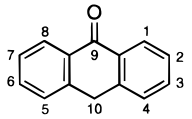
Anthralin itself is only a moderate 5-LO inhibitor. In isolated bovine PMNL it inhibits the production of LTB_4 with an IC_{50} of 37 μM.[15] We found that the isomers **2a**−**d** of anthralin (Table 1), anthracenones with other functional groups than hydroxyl (**3**, Table 1), the *O*-alkylated or *O*-acylated derivatives **4a**−**e**,**g** (Table 2), several of those compounds with additional functional groups at the anthralin nucleus (Table 3), and the phenols **7d**−**f** (Table 4) were slightly more potent than anthralin. In general, no straightforward structure−activity relationship could be defined, and only moderate enzyme inhibitors were obtained by these simple structure modifications.

Antioxidant and Prooxidant Determination. For the determination of the antioxidant potential of the compounds, we used the stable free radical DPPH.[33] Catechol **2c** (Table 1) is among the most reactive compounds with respect to this feature. Although **2a** has also a catechol moiety, its reactivity toward DPPH is reduced as compared to that of **2c**. This can be ascribed to hydrogen bonding between the 1-hydroxyl and the 9-keto group of the molecule. High reactivity is also observed for compounds with an electron-withdrawing group at position 2 of anthralin such as **5c**−**e** and the captodative-substituted 10-thio derivatives **6f**,**g** (Table 3). These compounds share a common feature in that the resulting C-10 radical of anthralin is conjugated not only with the 9-keto group of the anthracenone but also with the pertinent additional functional group. Similar results were observed in our earlier studies.[16,17] Moreover, compounds **5h**,**i** show dramatically increased reducing capability against DPPH.

Prooxidant properties of the compounds were defined by the deoxyribose assay, which is a sensitive test for the production of hydroxyl radicals.[34] The release of malondialdehyde (MDA) is indicative of oxygen-radical formation. With the exception of compounds **2a**, **3b**, **4a**, **5c**,**g**, and **6f**,**g**, the examined derivatives are either weaker generators of hydroxyl radicals than anthralin or devoid of prooxidant properties. It may be concluded that prooxidant properties do not necessarily result in potent antiproliferative activity. Thus, 1-hydroxy-9(10*H*)-anthracenone (**3b**; Table 1) and aldehyde **5c** (Table 3) are potent generators of hydroxyl radicals but not active against cell growth at concentrations up to 5 μM. On the other hand, only one compound, anthralin triacetate **4f** (Table 2), is completely devoid of prooxidant properties in the deoxyribose assay and is also a potent inhibitor of cell growth. However, **4f** is metabolized to parent **1** under our assay conditions.

Antiproliferative Activity. All the analogues and derivatives were assayed for their ability to inhibit

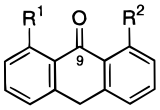
Table 1. Isomers of Anthralin and Analogues with Replaced Hydroxyl Groups



compd	R	log P^a	$k_{DPPH}{}^b$ (M^{-1} s^{-1})	DD (•OH)^c	IC$_{50}$ (μM) 5-LO^d	IC$_{50}$ (μM) AA^e	LDH^f (mU)
1	anthralin, 1,8-$(OH)_2$	4.23	24.2 ± 4.2	2.89 ± 0.14	37	0.6	294
2a	1,2-$(OH)_2$	3.19	41.5 ± 3.8	3.52 ± 0.14	4	1.9	ND
2b	1,5-$(OH)_2$	3.51	13.5 ± 2.4	1.28 ± 0.13	10	4.3	191
2c	3,4-$(OH)_2$	3.81	>100	1.19 ± 0.05	2	>5	144^g
2d	4,5-$(OH)_2$	2.17	<2	0.40 ± 0.05	4	>5	144^g
3a	9(10*H*)-anthracenone	3.46	<2	0.35 ± 0.02	2	>5	165
3b	1-OH	3.99	9.6 ± 0.5	2.95 ± 0.20	13	>5	170
3c	1,8-Cl_2	3.51	<2	<0.3	4	>5	ND
3d	1-NH_2	2.45	50.5 ± 4.2	0.52 ± 0.03	28	>5	ND
3e	4-OH, 5-OMe	2.60	<2	0.87 ± 0.07	2	>5	141^g
3f	4,5-$(OMe)_2$	3.68	<2	<0.3	8	>5	137^g
3g	4,5-$(OBn)_2$	5.73	3.2 ± 0.7	<0.3	ND	>5	ND
3h	4,5-Cl_2	5.05	3.4 ± 0.6	<0.3	ND	2.1	ND
3i	4-NH_2	2.46	15.3 ± 1.4	0.76 ± 0.11	20	>5	ND

^{a} Experimentally determined partition coefficients.[15] ^{b} Reducing activity against 2,2-diphenyl-1-picrylhydrazyl with equimolar amount of test compound.[15] ^{c} 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, $P < 0.01$).[15] ^{d} Inhibition of LTB_4 biosynthesis in bovine PMNL. Inhibition was significantly different with respect to control; $N = 3$ or more, $P < 0.01$. Nordihydroguaiaretic acid (NDGA) was used as the standard inhibitor (IC$_{50}$ = 0.4 μM).[15] ^{e} Antiproliferative activity against HaCaT cells.[16] Inhibition of cell growth was significantly different with respect to control, $N = 3$, $P < 0.01$. ^{f} Activity of LDH (mU) release in HaCaT cells after treatment with 2 μM test compound ($N = 3$, SD < 10%).[17] ^{g} Values are not significantly different with respect to vehicle control. ND = not determined.

Table 2. Anthralin Analogues with Substituted Oxygen Functions



compd	R^1, R^2	log P^a	$k_{DPPH}{}^b$ (M^{-1} s^{-1})	DD (•OH)^c	IC$_{50}$ (μM) 5-LO^d	IC$_{50}$ (μM) AA^e	LDH^f (mU)
1	anthralin, $R^1 = R^2 = OH$	4.23	24.2 ± 4.2	2.89 ± 0.14	37	0.6	294
4a	$R^1 = OH$, $R^2 = OAc$	3.25	16.1 ± 3.1	2.90 ± 0.40	6	0.8	243
4b	$R^1 = OH$, $R^2 = Bz$	3.99	39.6 ± 0.9	<0.3	27	4.3	ND
4c	$R^1 = OH$, R^2 = 2-AcOBz	3.49	16.7 ± 0.9	<0.3	12	3.7	ND
4d	$R^1 = OH$, $R^2 = OMe$	3.44	4.6 ± 0.8	2.54 ± 0.23	12	3.1	179
4e	$R^1 = R^2 = OAc$	2.40	3.6 ± 0.9	0.58 ± 0.04	9	0.7	314
4f	1,8,9-$(OAc)_3$-anthracene	2.02	<2	<0.3	>30	0.5	232
4g	1,8,9-$(OMe)_3$-anthracene	4.10	<2	<0.3	10	>5	165
4h	$R^1 = R^2 = OMe^h$	3.88	ND	<0.3	>30	>5	144^g
4i	$R^1 = R^2 = OH$, 9-NH^i	4.72	2.7 ± 0.4	0.44 ± 0.04	>30	0.5	137^g

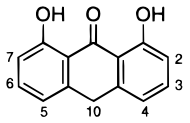
$^{a-g}$ See footnotes of Table 1. ^{h} Compound exists in the tautomeric anthracenol form.[9] ^{i} 1,8-Dihydroxy-9(10*H*)-anthracenimine.

keratinocyte proliferation, which is most important for prediction of antipsoriatic activity. As a model of epidermal hyperproliferation in psoriasis we used HaCaT cells, a rapidly multiplying human keratinocyte line. Proliferation of the keratinocytes was determined directly by counting the dispersed cells under a phase-contrast microscope after 48 h of treatment.

As stated earlier, anthralin is a potent inhibitor of the growth of HaCaT cells with an IC$_{50}$ in the 10^{-7} M range. A summary of the major structure−activity relationships for inhibition of keratinocyte proliferation is as follows. Transposition of hydroxyl groups at positions 1 and 8 to other positions (**2a**−**d**) or replacement of one or both of these groups by hydrogen (**3a**,**b**), chloro (**3c**,**h**), amino (**3d**,**i**), methoxy (**3e**,**f**, **4d**,**h**), or benzyloxy (**3g**) all results in compounds with little or no activity (Tables 1 and 2). Accordingly, anthrarobin (**2c**) and isoanthralin (**2d**) are inactive. Methylation of only one of the oxygens (**4d**) strongly reduces potency, while di- or trimethylation (**4h**,**g**) eliminates activity, presumably because the latter compounds exist in the tautomeric anthracene forms. Compounds with substituted oxygen functions are active only if these functions were esterified (**4a**−**c**,**e**,**f**), likely dependent on hydrolytic or enzymatic removal of the ester groups to give the parent **1**. In support of this, acetates **4a**,**e**,**f** have been reported to be prodrugs, which are transformed to the parent **1**.[35] However, replacement of the 9-keto by an imino function (**4i**) preserves the antiproliferative activity of **1**, documenting the requirement of a hydrogen bond acceptor *peri* to the hydroxyl groups.

Furthermore, introduction of simple substituents or functional groups into position 2 (**5a**−**e**), 3 (**5f**,**h**), or 10 (**6a**−**n**) of the anthralin pharmacophore results in either total loss or reduction of potency, and the IC$_{50}$ values shown in Table 3 document that there is no absolute requirement for either an electron-withdrawing or electron-donating function in these positions. The dimer **6o** is an exception and has already been reported to inhibit keratinocyte proliferation in earlier studies.[36,37] Further exceptions are compounds **5g**,**i**, which are as potent as **1**, both with an IC$_{50}$ value of 0.7 μM. However,

Table 3. Anthralin Analogues Bearing Simple Substituents



compd	R	log P^a	$k_{DPPH}{}^b$ (M^{-1} s^{-1})	DD (•OH)^c	IC_{50} (μM) 5-LO^d	IC_{50} (μM) AA^e	LDH^f (mU)
1	anthralin	4.23	24.2 ± 4.2	2.89 ± 0.14	37	0.6	294
5a	2-Me	4.99	17.2 ± 3.5	1.13 ± 0.09	>30	1.5	126^g
5b	2-*n*-C_4H_9	5.89	6.5 ± 0.4	<0.3	>30	>5	ND
5c	2-CHO	4.06	>100	3.44 ± 0.41	6	>5	ND
5d	2-COC_2H_5	4.59	> 100	2.24 ± 0.16	6	3.3	ND
5e	2-CN	3.16	>100	2.14 ± 0.26	8	4.2	ND
5f	3-Me	4.80	15.3 ± 1.9	2.35 ± 0.20	>30	1.5	211
5g	3-CH_2OH	3.37	20.1 ± 3.2	3.96 ± 0.34	30	0.7	376
5h	3-COOH	2.96	>100	1.04 ± 0.13	3	>5	ND
5i	3-CO_2Me	4.54	>100	1.09 ± 0.23	>30	0.7	340
5j	aloin	1.83	<2	<0.3	>30	>5	ND
5k	3-OH, 6-Me	3.90	21.4 ± 3.7	2.04 ± 0.16	14	>5	114^g
5l	harunganol A	6.56	8.2 ± 0.8	<0.3	25	>5	ND
5m	harunganol B	8.01	3.1 ± 0.1	< 0.3	>30	>5	ND
6a	10-Br	3.94	12.7 ± 1.4	<0.3	>30	>5	ND
6b	10-Et	4.37	<2	0.64 ± 0.05	>30	>5	161
6c	10-OH	2.91	<2	0.58 ± 0.04	>30	>5	ND
6d	10-OMe	3.96	ND	1.46 ± 0.03	>30	1.1	146^g
6e	10-OBn	4.25	ND	1.90 ± 0.02	>30	3.8	ND
6f	10-S-Et^h	2.97	>100	4.23 ± 0.14	>30	1.4	ND
6g	10-SCH_2Ph^h	4.87	>100	3.73 ± 0.36	>30	1.4	ND
6h	10-Bn	4.50	<2	0.35 ± 0.05	>30	>5	135^g
6i	10-CHO^i	2.79	<2	<0.3	7	1.9	124^g
6j	10-$COCH_3$	3.49	17.6 ± 3.5	1.47 ± 0.16	18	2.6	317
6k	10-COC_4H_9	4.12	7.0 ± 0.6	<0.3	>30	4.4	214
6l	10-Bz	3.81	ND	1.24 ± 0.17	4	>5	ND
6m	10,10-$(Bn)_2$	4.33	<2	<0.3	>30	>5	167
6n	10-=$CHNMe_2$	3.18	16.2 ± 0.4	<0.3	21	2.1	ND
6o	anthralin dimer	5.01	<1	<0.3	>30	0.6	263
6p	hypericin	5.02	3.66	<0.3	>30	1.7	159
6q	danthron	4.16	<2	<0.3	>30	>5	170

$^{a-g}$ See footnotes of Table 1. ^{h} Data were taken from ref 17. ^{i} Compound exists in the hydroxymethylene (enol) form.[13]

Table 4. Anthralin Analogues with Removed Structural Elements

compd	log P^a	$k_{DPPH}{}^b$ (M^{-1} $s^{-1)}$)	DD (•OH)^c	IC_{50} (μM) 5-LO^d	IC_{50} (μM) AA^e	LDH^f (mU)
1, anthralin	4.23	24.2 ± 4.2	2.89 ± 0.14	37	0.6	294
7a, anthracene	4.49	ND	<0.3	>30	>5	131^g
7b, 9,10-dihydroanthracene	4.06	ND	<0.3	>30	>5	ND
7c, 2,2′-dihydroxybenzophenone	2.66	2.1 ± 0.1	<0.3	>30	>5	ND
7d, juglone	2.74	6.6 ± 0.1	<0.3	17	0.8	384
7e, naphthazarin	3.70	41.5 ± 2.8	0.33 ± 0.03	3	0.7	247
7f, pyrogallol	1.19	>100	1.38 ± 0.30	4	>5	ND

$^{a-g}$ See footnotes of Table 1.

it should be stated that preparation of antiproliferative active analogues of anthralin is quite possible, if the 1,8-dihydroxy-9(10*H*)-anthracenone pharmacophore is preserved and appropriate substituents are introduced into position 2, 3,[16,18] or 10[15,17] of the parent molecule.

Of the natural products with an intact 1,8-dihydroxy-9(10*H*)-anthracenone structure, the anthracenones of aloe emodin (**5g**) and rhein methyl ester (**5i**) exhibit potent activity. This lends support to the traditional use of some anthracenone-containing plants for the treatment of psoriasis. The active principal of goa powder, chrysarobin (**5f**), which had been used earlier in the treatment of psoriasis and had been replaced by anthralin,[38] is also active but somewhat less potent than its synthetic substitute. By contrast, the naturally occurring harunganol A and B[39] (**5l**,**m**) were inactive, an observation which may be due to less facile penetration through the cell membrane by these more lipophilic compounds. Partition coefficients, as a measure of lipophilicity of the compounds, were determined by HPLC and are expressed as log *P* values (Tables 1−4). From these data it is obvious that with log *P* values of 6.56 and 8.01, respectively, **5l**,**m** are substantially more lipophilic than the other anthracenones. In general, there is no clear relationship found between the antiproliferative activity of the test compounds and lipophilicity. On one hand the compounds exhibit a wide range of log *P* values (1.19−8.01), while on the other hand, with the exception of prodrugs **4e**,**f**, the highly potent analogues (IC_{50} value < 1 μM) exhibit log *P* values between 2.74 (**7d**) and 5.01 (**6o**).

Also, where the 1,8-dihydroxy-9-keto groups are intact, but both hydrogen atoms at C-10 are substituted by alkyl groups or oxygen (**6m**,**q**) or the middle ring is opened (**7c**, Table 4), antiproliferative activity is lost. Replacement of the C-10 hydrogens by a (dimethylamino)methylene group (**6n**), however, retained activity, although decreased as compared to **1**. Surprisingly, the

change from a tricyclic anthracenone to a bicyclic naphthalenedione produces active compounds (**7d**,**e**), as shown in Table 4. Since analogues **3b**, **6q**, and **7c**, where intramolecular hydrogen bonding between the hydroxyl and the keto group is enabled, are inactive as antiproliferative agents, this structural feature alone cannot account for the activity of **7d**,**e**. The monocyclic **7f** is inactive.

Cytotoxicity. The results of the cytotoxicity studies document that all compounds with high antiproliferative activity show increased membrane damage. This is evidenced by release of lactate dehydrogenase activity into the culture medium,[40] which significantly exceeded that of the vehicle control. The activity was about in the same order of magnitude as that of anthralin.

Conclusions

In vivo studies indicate that LTB_4 promotes keratinocyte growth in guinea pig skin,[27] and an in vitro study with human keratinocytes showed a statistically significant mitogenic effect, however weak, of LTB_4 and other leukotrienes.[26] Although many of the analogues inhibited 5-LO at micromolar concentrations, only few had the potency of the parent compound **1** to inhibit keratinocyte growth. In general, results from the two assays did not show a reasonable correlation. Thus, compounds **2c**,**d**, **3a**−**f**,**i**, **4g**, **5c**,**h**,**k**,**l**, and **6l** display activity in the 5-LO assay but lack antiproliferative activity. Conversely, derivatives **4f**,**i**, **5i**, and **6o** are inactive against the 5-LO enzyme but inhibit cell growth in the 10^{-7} M range.

In light of these findings it is suggested that 5-LO inhibition alone is not sufficient for potent antiproliferative action. Although clinical results with 5-LO inhibitors such as lonapalene were encouraging in that a reduction of LTB_4 was seen before the observed clinical improvement of psoriasis,[41] additional or other biological properties of the antiproliferative active compounds may be responsible for their efficacy. The corresponding IC_{50} values for lonapalene obtained for inhibition of 5-LO and cell growth in our assays were 0.5 and 3.2 μM, respectively.[16] Nevertheless, potent 5-LO inhibition may be appropriate to manage at least the inflammatory component of psoriasis.

The aim of this study was to elucidate the structure−activity relationships of simple analogues of anthralin, to further delineate the nature of the minimum requirements of the pharmacophore, initially formulated as **3b**.[7] A striking feature of the results given in Tables 1 and 2 is the marked loss or decrease in antiproliferative activity when any of the 1,8-dihydroxy substituents of anthralin is modified, except for the esters **4a**,**e**,**f**, which can be transformed to the parent anthralin.[35] The postulated "minimum structure for antipsoriatic activity", compound **3b**, is not active at 5 μM. Also, upon movement of the second hydroxyl group to other positions, a significant drop in activity is observed, while any other changes result in antiproliferative inactive compounds. There is also an absolute requirement of a hydrogen bond acceptor such as a keto or an imino function in the 9-position, i.e., *peri* to the hydroxyl groups.

In addition to these conclusions we have also identified naphthalenediones **7d**,**e**, with a *peri* hydroxyl group, as a pharmacophore with potent antiproliferative action against keratinocyte growth.

Whatever the molecular mechanism of the antiproliferative action of anthralin and wherever its locus, the results described herein indicate that it is sensitive to the slightest modification in the structure of anthralin and that active analogues can only be made if the anthralin moiety itself is retained. An early decision in our attempts to develop new therapeutically effective antipsoriatic drugs was to keep the 1,8-dihydroxy oxygens of the anthracenone unchanged,[15−18] because these structural parts may be important for antipsoriatic activity. Thus, it appears that the proposed structural pattern hypothesis has been substantiated by the experimental results obtained from the present paper. This unique property should definitely be noted in future drug design.

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) with CH_2Cl_2 as eluant, unless otherwise stated. ^{1}H NMR spectra were recorded with a Varian EM 390 (90 MHz), Bruker Spectrospin WM 250 (250 MHz), or Bruker ARX 400 (400 MHz) spectrometer using tetramethylsilane as an internal standard. Fourier-transform IR spectra (KBr) were recorded on a Nicolet 510M FTIR spectrometer. Mass spectra (EI, unless otherwise stated) were obtained on a Varian MAT CH5 spectrometer (70 eV).

The following anthracenones were prepared by previously described procedures: **1**,[19] **2a**,[19] **2b**,[42] **2c**,[19] **2d**,[9] **3b**,[43] **3c**,[19] **3d**,[44] **3e**−**i**,[19] **4a**,[45] **4b**,**c**,[13] **4d**,[19] **4e**,**f**,[45] **4g**,[35] **4h**,[9] **5f**,[46] **5g**,[47] **5h**,**k**,[46] **6a**,[20] **6b**,[48] **6c**,[49] **6d**,[50] **6f**,**g**,[17] **6h**,[15] **6j**,[49] **6k**,[51] **6l**,[13] **6m**,[15] **6n**,[13] and **6o**.[46] Harunganol A and B (**5l**,**m**) were a kind gift of Dr. M. Iinuma (Gifu Pharmaceutical University, Japan).[39] All other compounds were commercial materials.

1,8-Dihydroxy-9(10*H*)-anthracenimine (4i). To a stirred solution of methanolic ammonia (300 mL), saturated at 0 °C, was added dropwise **1** (1.13 g, 5 mmol) in dry CH_2Cl_2 (75 mL). The solution was stirred for 1 h at 0 °C, stored at 4 °C for 24 h, and evaporated. The residue was purified by chromatography using CH_2Cl_2/hexane (8/2) to provide yellow crystals (10%): mp 205−207 °C; ^{1}H NMR (400 MHz, *N*,*N*-dimethylformamide-d_6) δ 11.62 (s, 2H), 7.54 (t, J = 7.95 Hz, 2H), 6.95 (d, J = 8.4 Hz, 2H), 6.64 (d, J = 7.5 Hz, 2H), 4.93 (s, 1H); FTIR 1630 cm^{-1}; MS (PI−FD, CH_2Cl_2) *m*/*z* 225. Anal. $C_{14}H_{11}NO_2$ (C, H).

1,8-Dihydroxy-2-methyl-9(10*H*)-anthracenone (5a). To a solution of 1-hydroxy-8-methoxy-2-methyl-9,10-anthracenedione[52] (0.19 g, 0.70 mmol) in glacial acetic acid (20 mL) heated to reflux was added, dropwise over 3 h, a solution of $SnCl_2$ (2.5 g, 13.19 mmol) 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 (58%): mp 129 °C; ^{1}H NMR (90 MHz, $CDCl_3$) δ 12.72 (s, 1H), 12.45 (s, 1H), 7.66−7.32 (m, 2H), 7.03−6.77 (m, 3H), 4.32 (s, 2H), 2.32 (s, 3H); FTIR 1621 cm^{-1}. Anal. $C_{15}H_{12}O_3$ (C, H).

2-*n*-Butyl-1,8-dihydroxy-9(10*H*)-anthracenone (5b): prepared from 2-*n*-butyl-1-hydroxy-8-methoxy-9,10-anthracenedione[53] as described for **5a** to give yellow crystals (82%); mp 105−106 °C; ^{1}H NMR (90 MHz, $CDCl_3$) δ 12.52 (s, 1H), 12.27 (s, 1H), 7.44−6.64 (m, 5H), 4.17 (s, 2H), 2.77−2.50 (m, 3H), 1.78−1.24 (m, 4H), 1.00−0.88 (m, 3H); FTIR 1618 cm^{-1}; MS *m*/*z* 282 (89), 239 (100). Anal. $C_{18}H_{18}O_3$ (C, H).

9,10-Dihydro-1,8-dihydroxy-9-oxo-2-anthracenecarbaldehyde (5c): prepared from **8c** as described for **5a** to give red-brown needles (61%); mp 200 °C dec; ^{1}H NMR (90 MHz, DMSO-d_6) δ 13.03 (s, 1H), 11.92 (s, 1H), 10.48 (s, 1H), 8.12−6.70 (m, 5H), 4.38 (s, 2H); FTIR 1686, 1607 cm^{-1}. Anal. $C_{15}H_{10}O_4$ (C, H).

1,8-Dihydroxy-2-[1-(1-oxopropyl)]-9(10*H*)-anthracenone (5d): prepared from 1-hydroxy-8-methoxy-2-[1-(1-oxopropyl)]-9,10-anthracenedione[54] as described for **5a** to give

an orange-red powder (31%); mp 175 °C; ^{1}H NMR (250 MHz, DMSO-d_6) δ 13.44 (s, 1H), 11.94 (s, 1H), 7.52−6.44 (m, 5H), 4.53 (s, 2H), 3.05 (q, J = 7.14 Hz, 2H), 1.12 (t, J = 6.99 Hz, 3H); FTIR 1636, 1619 cm^{-1}. Anal. $C_{17}H_{14}O_4$ (C, H).

9,10-Dihydro-1,8-dihydroxy-9-oxo-2-anthracenecarbonitrile (5e): prepared from **8d** as described for **5a** to give yellow needles (31%); mp 185 °C dec; ^{1}H NMR (90 MHz, $CDCl_3$) δ 13.06 (s, 1H), 11.83 (s, 1H), 7.56−6.78 (m, 5H), 4.36 (s, 2H); FTIR 2224, 1617 cm^{-1}. Anal. $C_{15}H_9NO_3$ (C, H, N).

Rhein anthracenone methyl ester (5i): prepared from rhein methyl ester[55] as described for **5a** to give a yellow powder (63%); mp 171−172 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 12.20 (s, 1H), 12.10 (s, 1H), 7.57−7.37 (m, 3H), 6.93 (s, 1H), 4.37 (s, 2H), 3.93 (s, 3H); FTIR 1725, 1619 cm^{-1}; MS *m*/*z* 284 (100). Anal. $C_{16}H_{12}O_5$ (C, H).

1,8-Dihydroxy-10-(phenylmethoxy)-9(10*H*)-anthracenone (6e). To a solution of **6a**[20] (305 mg, 1 mmol) and 0.1 mL of trifluoroacetic acid in dry CH_2Cl_2 (20 mL) was added dropwise a solution of benzyl alcohol (216 mg, 2 mmol) in dry CH_2Cl_2 (10 mL) under N_2. The reaction mixture was stirred at room temperature for 6 h, and the solvent was removed. Recrystallization from ethanol provided yellow crystals (18%): mp 102 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 12.18 (s, 2H), 7.62 (t, J = 8.0 Hz, 2H), 7.33−7.23 (m, 7H), 7.00 (d, J = 8.2 Hz, 2H), 5.87 (s, 1H), 4.15 (s, 2H); FTIR 1630 cm^{-1}; MS *m*/*z* 332 (3), 240 (100). Anal. $C_{21}H_{16}O_4$ (C, H).

9,10-Dihydro-1,8-dimethoxy-9,10-dioxo-2-anthracenecarbaldehyde (8b). To a suspension of anhydrous $NaHCO_3$ (2.00 g) in dry DMSO (50 mL) at 40 °C (oil bath) was added **8a**[56] (2.0 g, 5.54 mmol). The oil bath was removed, and the mixture was stirred for 1 h. Then it was poured into a mixture of ice−water (500 g) and 37% HCl (20 mL) and stirred for an additional 15 min. The product was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic phase was washed with water (3 × 100 mL), dried over Na_2SO_4, and evaporated. The resulting residue was purified by chromatography to give yellow needles (60%): mp 227 °C dec; ^{1}H NMR (90 MHz, $CDCl_3$) δ 10.53 (s, 1H), 8.20−7.23 (m, 5H), 4.17 (s, 3H), 4.05 (s, 3H); FTIR 1694, 1679 cm^{-1}. Anal. $C_{17}H_{12}O_5$ (C, H).

9,10-Dihydro-1,8-dihydroxy-9,10-dioxo-2-anthracenecarbaldehyde (8c). To a suspension of **8b** (1.0 g, 3.38 mmol) in HOAc (25 mL) heated to reflux was added dropwise 62% HBr (5 mL), and the solution was refluxed for 3 h. The solution was cooled, water (20 mL) was added, and the mixture was allowed to stand for 30 min. The precipitate was filtered off, washed with water (50 mL), and dried at 50 °C. Purification by chromatography provided orange-yellow crystals (79%): mp 201 °C; ^{1}H NMR (90 MHz, $CDCl_3$) δ 12.71 (s, 1H), 11.89 (s, 1H), 10.62 (s, 1H), 8.25−7.26 (m, 5H); FTIR 1692, 1677, 1625 cm^{-1}. Anal. $C_{15}H_8O_5$ (C, H).

9,10-Dihydro-1,8-dimethoxy-9,10-dioxo-2-anthracenecarbonitrile (8d). A suspension of **8b** (0.85 g, 2.87 mmol) and hydroxylamine hydrochloride (0.24 g, 3.5 mmol) in formic acid (15 mL) was refluxed for 3 h. The solution was cooled, poured into a mixture of ice−water (200 mL), and then neutralized with 5% NaOH, and the product was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phase was washed with water (2 × 50 mL), dried over Na_2SO_4, and evaporated. The resulting residue was purified by chromatography to give yellow needles (14%): mp 230 °C dec; ^{1}H NMR (90 MHz, $CDCl_3$) δ 8.09−7.36 (m, 5H), 4.21 (s, 3H), 4.05 (s, 3H); FTIR 2240, 1673 cm^{-1}. Anal. $C_{17}H_{11}NO_4$ (C, H, N).

Biological Assay Methods. Determination of the reducing activity against 2,2-diphenyl-1-picrylhydrazyl,[33] degradation of 2-deoxy-*d*-ribose,[33] bovine PMNL 5-LO assay,[15] HaCaT keratinocyte proliferation assay,[16] and LDH release[17] were described previously in full detail.

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