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10-ω-PHENYLALKYL-9(10*H*)-ANTHRACENONES AS INHIBITORS OF KERATINOCYTE GROWTH WITH REDUCED MEMBRANE DAMAGING PROPERTIES

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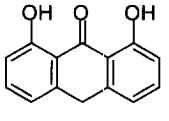
Abstract: Work aimed at further improving the benefit to risk ratio of the antipsoriatic agent anthralin has led to 10-ω-phenylalkyl-9(10*H*)-anthracenones, members of which are equally potent as inhibitors of the growth of HaCaT keratinocytes. In contrast to anthralin, induction of membrane injury is strongly reduced as documented by the release of LDH activity from cytoplasm of keratinocytes. © 1998 Elsevier Science Ltd. All rights reserved.

Psoriasis is now treated by drugs such as vitamin D analogs and retinoids, which serve only to abate this hyperproliferative skin disorder. Yet antipsoriatic anthracenones such as anthralin (**1**) clear the pathological features of psoriasis totally.[1,2] Because of their outstanding therapeutic properties, interest continues unabated in the development of novel analogues. Like most forms of current therapy of psoriasis the benefits of the drugs are limited by their undesirable proinflammatory effects on the skin or poor acceptability because of staining of the skin and clothing.[3] Therefore, the development of a topically active analogue which should obviate these drawbacks is highly desirable. The mode of action of anthracenones is thought to be mediated by oxygen radicals and anthralin metabolite radicals.[4]



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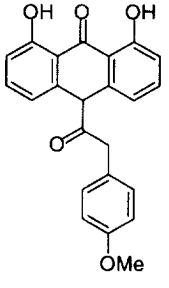

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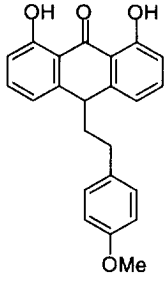

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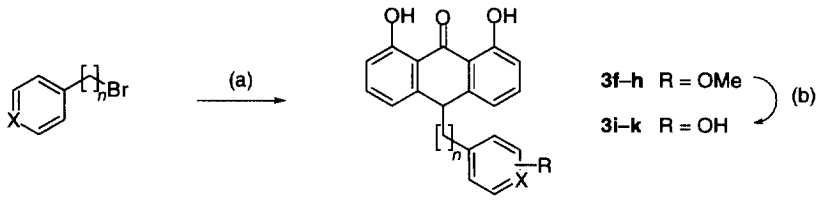
Antipsoriatic anthracenones exhibit three principal cellular effects: (1) interaction with DNA, (2) inhibition of various enzyme systems associated with cell proliferation and inflammation, and (3) redox reactions with the resulting alteration of mitochondrial functions and destruction of membrane lipids.[5] Oxygen radicals have been implicated in all these processes but are not necessarily dominant in these effects.[6] As the mechanism of the proinflammatory action of anthracenones is associated with the formation of oxygen radicals by these agents,[4] our strategy to overcome this problem was to modulate the oxygen-radical generating intensity, in particular by modifying the critical 10-position of the pharmacophore.[7-10] Structure–activity relationships of simple analogues of anthralin revealed a marked decrease in antiproliferative activity when any of the 1,8-dihydroxy substituents was modified.[11] However, synthesis of congeners in which one of the active methylene protons at C-10 of the anthracenone were replaced by suitable substituents has resulted in high activity in preclinical models.

Of the 10-phenylacyl series of antipsoriatic anthracenones, the 4-methoxyphenylacetyl analogue **2** is presently undergoing clinical evaluation as a topical treatment for psoriasis. Even though decomposition of 10-acyl analogues to the parent anthralin and further to polymers responsible for the darker shades of brown staining that occurs on the skin and clothes has been discarded, these compounds are slowly degraded to the corresponding anthracenedione. Also, staining of clothes after washing with alkaline detergents has been observed. Therefore, more stable analogues than 10-acyl anthrancenones are required.

In previous studies on 10-alkylated congeners no antipsoriatic activity was observed for the 10-ethyl analogue in clinical examination,[12] and the 10-benzyl analogue was inactive in an *in vitro* assay.[7] In order to elucidate further the structural requirements for *in vitro* activity of 10-alkylated anthracenones, we synthesized a series of 10-ω-phenylalkyl-1,8-dihydroxy-9(10*H*)-anthracenones and evaluated their reduced intensity of hydroxyl-radical formation, antiproliferative activity against human keratinocytes, and their reduced potential to induce membrane damage as compared to anthralin.

Scheme 1[a]



3a–h, m, n

[a]R, X, and *n* are defined in Table 1. Reagents: (a) anthralin, K_2CO_3, KI, acetone, Δ, N_2; (b) BBr_3, CH_2Cl_2, -78 °C, N_2.

Chemistry

Introduction of the 10-ω-phenylalkyl substituents onto the anthracenone nucleus was achieved by reaction of the appropriate phenylalkyl bromides[13] with anthralin in the presence of potassium carbonate in acetone (Scheme 1). The desired C-10 monoalkylated anthracenones **3a–3h,m,n** were separated from *O*-alkylated and dibenzylated products by column chromatography.[14] The phenolic analogues **3i–l** were prepared by cleavage of the corresponding methyl ethers **3f–h** with boron tribromide.[15]

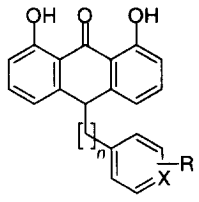
Biological Evaluation

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.[16] Accordingly, 10-ω-phenylalkyl anthracenones were evaluated for inhibition of the growth of HaCaT cells. Keratinocytes were also tested for their susceptibility for the action of the most potent members of this series on plasma membrane integrity, in order to confirm that inhibition of cell growth is not a result of membrane damage induced by prooxidants released from anthracenones. Finally, the prooxidant potential of the compounds was determined by deoxyribose degradation.[17]

Hydroxyl-Radical Generation. The deoxyribose assay is a sensitive test for the production of hydroxyl radicals.[18] The release of malondialdehyde (MDA) is a measure of this feature and reflects the prooxidant properties of the compounds. Table 1 shows that partially blocking the C-10 position of anthralin in general strongly reduces the intensity of hydroxyl-radical generation. Exceptions are the pyrogallol and pyridine analogues **3l** and **3b**, respectively.

Antiproliferative Activity. 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. While previous members of the 10-ω-phenylalkyl series[7] (**3a,c,d**) are inactive as inhibitors of 5-lipoxygenase and are also inactive against the growth of HaCaT keratinocytes at concentration up to 5 μM, introduction of a 4-methoxy group into the phenylethyl substituent (**3f**) retains the potent antiproliferative activity of anthralin. Addition of further methoxy groups (**3g,h**), however, decreases activity. The corresponding phenolic analogue **3i** of methoxy derivative **3f** is a much less potent inhibitor of keratinocyte growth, while the catechol and pyrogallol analogues **3k** and **3l**, respectively, are not active at 5 μM. This may be due to their decreased lipophilicity, log *P* values of 3.09 and 3.65 *versus* 5.07 for **3i**, which may be relevant for penetrating into the cells. This also documents that the potency of an anthracenone to generate hydroxyl radicals, as observed for **3l**, is not necessarily related to its antiproliferative action. Furthermore, it is interesting to note that exchanging the terminal phenyl ring with a 4-pyridine ring (**3b**) also results in improved antiproliferative activity.

Table 1. Antiproliferative Activity and Cytotoxicity against HaCaT Cells and Hydroxyl-Radical Generation of 10-ω-Phenylalkyl-1,8-dihydroxy-9(10*H*)-anthracenones



compd	n	X	R	log P^a	AA IC_{50} (μM)^b	LDH (mU)^c	$^{\bullet}OH$ μmol MDA/mmol deoxyribose^d
1				4.23	0.7	294	2.89 ± 0.14^e
2^f				4.20	1.1	143^g	< 0.2
3a	1	CH	H	4.50	> 5	150^g	0.35 ± 0.05^e
3b	1	N	H	3.47	1.8	179	1.25 ± 0.12^e
3c	1	CH	4-OMe	4.45	> 5	152^g	< 0.2
3d	1	CH	4-NO_2	4.16	> 5	142^g	< 0.2
3e	2	CH	H	5.27	> 5	ND	< 0.2
3f	2	CH	4-OMe	5.07	0.9	129^g	< 0.2
3g	2	CH	3,4-$(OMe)_2$	4.55	3.0	ND	< 0.2
3h	2	CH	3,4,5-$(OMe)_3$	4.37	4.1	ND	0.97 ± 0.22^e
3i	2	CH	4-OH	4.10	3.8	136^g	0.40 ± 0.04^e
3k	2	CH	3,4-$(OH)_2$	3.65	> 5	ND	0.56 ± 0.12^e
3l	2	CH	3,4,5-$(OH)_3$	3.09	> 5	225	2.30 ± 0.04^e
3m	3	CH	H	5.51	> 5	130^g	< 0.2
3n	4	CH	H	5.72	> 5	ND	< 0.2

^{a}Experimentally determined partition coefficient.[7] ^{b}Antiproliferative activity against HaCaT cells. Inhibition of cell growth was significantly different with respect to that of the control (N = 3, P < 0.01). ^{c}Activity of LDH (mU) release in HaCaT cells after treatment with 2 μM test compound; values are significantly different with respect to vehicle control (N = 3, SD < 10%, P < 0.01). ^{d}Deoxyribose degradation as a measure of hydroxyl-radical generation. Indicated values are μmol of malondialdehyde/mmol of deoxyribose released by 75 μM test compound (controls < 0.1). ^{e}Values are significantly different with respect to control; P < 0.01. ^{f}Reference 7. ^{g}Values are not significantly different with respect to vehicle control; P < 0.01. ND = not determined.

Integrity of the Plasma Membrane. Cytotoxicity of the cell cultures was assessed by the activity of lactate dehydrogenase (LDH) released into the culture medium.[8] As a result of its potential to generate oxygen radicals, anthralin is an inducer of lipid peroxidation in biological membranes.[19] The results of Table 1 indicate that anthralin along with the most potent hydroxyl-radical generators of the 10-ω-phenylalkyl series, pyridine analogue **3b** and pyrogallol **3l**, are able to induce considerable plasma membrane damage at concentrations of 2 μM. Anthralin itself shows a twofold increase in LDH activity as compared to controls, and the amount of LDH release after treatment with **3b** and **3l** is directly related to the prooxidant potential of these analogues. By contrast, LDH release after treatment of HaCaT cells with the most potent inhibitor of cell growth of the phenylalkyl series, **3f**, does not exceed the control values. This documents that the activity of **3f** is due to cytostatic rather than cytotoxic effects and further confirms that appropriate structural modification of anthralin leads to control of the release of hydroxyl radicals resulting in reduced oxidative damage to membrane without sacrifice of biological activity.

Conclusions

10-ω-Phenylalkyl-substituted anthracenones were synthesized and evaluated in various assays. The first analogues of this series made in our laboratories gave disappointing *in vitro* activity. However, proper selection of the phenylalkyl substituent such as 4-methoxyphenylethyl (**3f**) or 4-pyridylmethyl (**3b**) leads to potent inhibitors of the growth of HaCaT keratinocytes. Overall, we found that the 10-(4-methoxyphenyl)acetyl fragment of the lead compound **2** can be successfully replaced by an analogous and more stable 10-(4-methoxyphenyl)ethyl moiety without sacrificing antiproliferative activity. Moreover, as a consequence of the strongly reduced intensity of hydroxyl-radical generation **3f** does not interfere with the functioning of cell membrane by causing leakage of cytoplasm through it. This results in an improved ratio of antiproliferative activity to cytotoxicity as compared to the antipsoriatic anthralin.

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14. **General Procedure for the Preparation of 1,8-Dihydroxy-10-(ω-phenylalkyl)-9(10*H*)-anthracenones 3a–h,m,n. 1,8-Dihydroxy-10-[2-(4-methoxyphenyl)ethyl]-9(10*H*)-anthracenone (3f).** Anthralin (**1**, 1.35 g, 6 mmol) and dry K_2CO_3 (2.07 g) were suspended in absolute acetone (75 mL) under N_2. 1-(2-Bromoethyl)-4-methoxybenzene[20] (1.51 g, 7 mmol) and catalytic amounts of potassium iodide were added and the mixture was refluxed for 12 h under N_2. Then the mixture was cooled, treated with water (300 mL) and 2 N sulphuric acid, and extracted exhaustively with CH_2Cl_2 (5 x 50 mL). The combined organic extracts were washed with water, dried over Na_2SO_4, and then evaporated. The residue was purified by chromatography (SiO_2; CH_2Cl_2/hexane 75/25) to afford **3f** as a yellow powder (16%): mp 86–87 °C (hexane); ^{1}H-NMR ($CDCl_3$) δ 12.27 (s, 2H), 7.60–6.77 (m, 10H), 4.30 (t, 1H, J = 4 Hz), 3.73 (s, 3H), 2.17–2.13 (m, 4H); FTIR 1636 cm^{-1} (CO···HO). Anal. ($C_{23}H_{20}O_4$) C, H.
15. **General Procedure for the Cleavage of Methyl Ethers (3i–l). 1,8-Dihydroxy-10-[2(4-hydroxyphenyl)ethyl]-9(10*H*)-anthracenone (3i).** A solution of **3f** (0.36 g, 1 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise to a solution of BBr_3 (5 mmol) in CH_2Cl_2 (20 mL) at -78 °C under N_2. The mixture was allowed to warm to room temperature and stirred for 24 h. Excess water was added and the mixture was extracted with ether (3 x 50 mL). The ether phase was dried over Na_2SO_4 and evaporated, and the residue purified by chromatography (SiO_2; CH_2Cl_2/ether 90/10) to afford **3i** as a yellow powder (40%): mp 192 °C; ^{1}H-NMR ($CDCl_3$) δ 12.22 (s, 2H), 8.67 (s, 1H), 7.63–6.54 (m, 10H), 4.32 (t, 1H, J = 5 Hz), 2.13–2.07 (m, 4H); FTIR 3411 (OH), 1636 cm^{-1} (CO···HO). MS *m/z* 346 (18), 226 (100). Anal. ($C_{22}H_{18}O_4$) C, H.
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