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Original article

# Structure–activity relationship studies of acridones as potential antipsoriatic agents. 2. Synthesis and antiproliferative activity of 10-substituted hydroxy-10*H*-acridin-9-ones against human keratinocyte growth

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ABSTRACT

A series of 10-substituted hydroxy-10*H*-acridin-9-ones were synthesized and studied as potential antipsoriatic agents. The antiproliferative activity of the novel derivatives, which can be considered as aza-analogues of the antipsoriatic drug anthralin, was determined using the human keratinocyte cell line HaCaT. Structure–activity relationships with respect to the nature of the N-substituent at the acridone scaffold were delineated. Release of lactate dehydrogenase (LDH) was used to exclude non-specific cytotoxic effects. As compared to anthralin, N-substitution of the acridone scaffold in the target compounds provided agents devoid of radical producing properties, which was documented by their ineffectiveness to interact with the free radical 2,2-diphenyl-1-picrylhydrazyl. This was in excellent agreement with the data obtained from the LDH assay in which the novel compounds did not induce membrane damage. Benzyl substitution at the 10-position yielded keratinocyte growth inhibitory activity in the low micromolar range. The most potent inhibitor of keratinocyte hyperproliferation was compound **8a** having an N-methyl group and a 1,3-dihydroxy arrangement at the acridone scaffold, with an $IC_{50}$ value comparable to that of anthralin.



## 1. Introduction

The acridone alkaloids constitute a small group of naturally occurring compounds produced exclusively by higher plants belonging to the order Rutales [1]. Because of their demonstrated antiproliferative activity, interest continues unabated in the synthesis of related synthetic analogues of the tetracyclic acridone alkaloid acronycine (**1**, Chart 1) [2–6] and 10*H*-acridin-9-ones possessing antitumor or other interesting biological activities [7–12]. Acridones can be considered as 10-aza-analogues of the anthrone class of topical agents against psoriasis, an inflammatory and scaling skin disease, which is mainly characterized by hyperproliferation of keratinocytes [13]. Owing to the proinflammatory and staining effects of antipsoriatic anthrones such as anthralin (dithranol, 1,8-dihydroxy-10*H*-anthracen-9-one, **2**), this drug is best used in hospital or day treatment centers [14], and there is need for analogues with improved therapeutic properties. Several studies have suggested that the side effects of the anthrones are associated with their capability to generate oxygen radicals [15,16], and the methylene moiety at C-10 has been recognized as a key site of radical formation [17,18]. Indeed, appropriate chemical modification of the critical C-10 of the anthrone pharmacophore provided agents with diminished oxygen radical formation [19–22].

In this direction, our interest has focused on the synthesis of 10-aza-analogues of anthrones in order to identify improved antipsoriatic agents that act via the keratinocyte as the primary target cell [23]. The antiproliferative activity of anthralin is intimately effected by the nature and position of the substituents at the anthrone nucleus. Both hydroxy groups *peri* to the keto group are required for high activity [24]. In our recent study [23] we have shown that structure–activity relationships (SAR) for N-unsubstituted acridone derivatives did not follow those of the antipsoriatic anthrones. Surprisingly, the aza-analogue of anthralin, 1,8-dihydroxy-10*H*-acridin-9-one (**3**), was only marginally active. However, acridone **4** comprising a 1,3-dihydroxy-substitution pattern was the most active analogue, with keratinocyte growth inhibitory potency comparable to anthralin. In this communication, we expand on this previous report and discuss SAR for modification of the substituent at the acridone nitrogen atom.

We have tested the novel 10-substituted acridones as inhibitors of keratinocyte hyperproliferation, which is one of the hallmarks of

*Abbreviations:* DPPH, 2,2-diphenyl-1-picrylhydrazyl; LDH, lactate dehydrogenase; RT, room temperature; SAR, structure–activity relationships.

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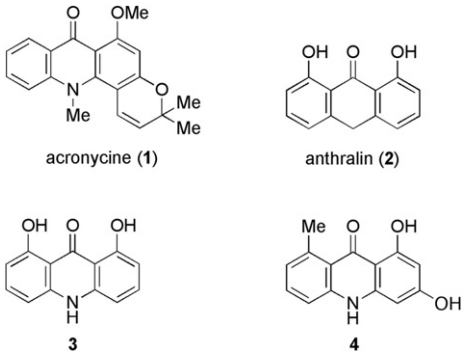



**Chart 1.** Structures of acronycine (**1**), anthralin (**2**), aza-analogous anthralin (**3**), and acridone derivative **4**.

psoriasis. We also evaluated their capability to interact with a stable free radical by use of 2,2-diphenyl-1-picrylhydrazyl (DPPH). Furthermore, release of lactate dehydrogenase (LDH) was measured to exclude cytotoxic effects mediated by membrane damage.

## 2. Chemistry

All target compounds were easily prepared from N-unsubstituted acridones. Thus, 1-hydroxyacridone (**5**) was N-alkylated with an appropriate alkyl halogenide and potassium carbonate according to Scheme 1. The preparation of **6a** and **6d** was accompanied by side products, O-alkylated **6aa** and **6dd**, respectively. Using benzyl bromide as an alkylating agent, also 2-benzylated **6ddd** was obtained by aromatic electrophilic substitution. The structure was confirmed by NOE experiments. On irradiation at the frequency of the $NCH_2$ protons (5.74 ppm), a positive NOE was observed for the signals at 6.96 (H-4) and 7.64 ppm (H-5), whereas on irradiation at the frequency of the $ArCH_2$ protons (3.94 ppm), a positive NOE was observed for the signal at 7.51 (H-3) and also 14.91 ppm (OH) was detectable.

In the case of 1,3-dihydroxyacridone (**7**), also the non-hydrogen-bonded hydroxy group in 3-position was alkylated to give acridones **8aa**–**8ee**, which were then smoothly converted into the final 1,3-dihydroxyacridones **8a**–**8e** by ether cleavage with 48% hydrobromic acid (Scheme 2). Acridones **8aa**, **8bb** and **8dd** were also isolated and purified. Acridones **9b**–**9e** were prepared by reaction of unsubstituted acridone (**9a**) with the approriate alkyl halogenides (Scheme 3). Finally, acetylation of **9a** or 1-hydroxylated **5** was performed in the presence of sodium hydride as described by Lewis [25]. However, in both cases only the acetoacetyl-substituted acridones **11** and **12**, respectively, were obtained (Scheme 4).

## 3. Biological assay methods

In the present study, we used HaCaT keratinocytes [26] as a model for the highly proliferative epidermis in psoriasis. This rapidly dividing, non-transformed human cell line is a useful tool in identifying new topical antipsoriatic agents, and it was described as an extremely sensitive target for the antiproliferative action of the antipsoriatic drug anthralin [27]. Accordingly, we evaluated the effects of the novel acridones on the growth of cultured HaCaT cells, which was determined directly by counting the dispersed cells under a phase-contrast microscope after 48 h of treatment.

Keratinocytes were also tested for their susceptibility to the action of the most potent members of this 10-substituted acridone series on plasma-membrane integrity, to confirm that inhibition of keratinocyte growth was not a result of membrane damage. This was assessed by the activity of LDH released into the culture medium [19,27].

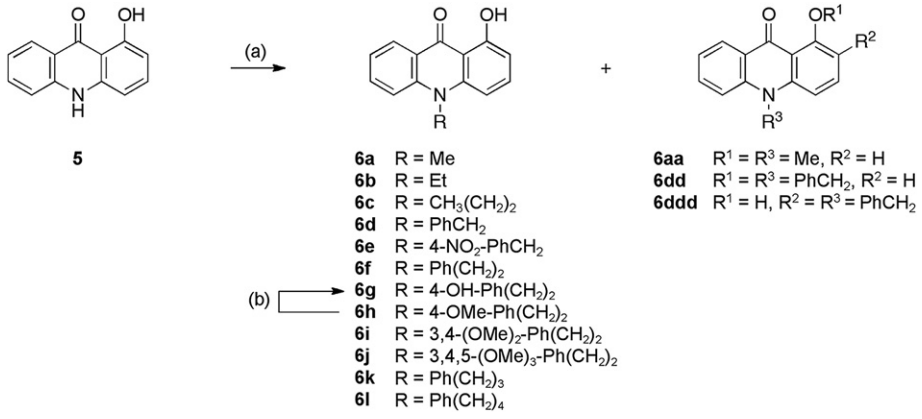
Furthermore, free radical production by anthralin and the resulting dimerization and polymerization of the molecule is associated with the instability of the drug as well as with undesired side effects such as staining and irritation of the skin [16]. In order to compare the capability of the acridones to produce radicals by loss of a hydrogen atom with that of anthralin, we determined the extent of the N-substituted acridones to scavenge radical intermediates by measuring spectrophotometrically the decrease of DPPH concentration [28] at 520 nm. When DPPH reacts with an electron-donating test compound (i.e., a hydrogen atom donor), it is reduced to the corresponding hydrazine, whereas the primary species derived from the test compound is a free radical.

## 4. Results and discussion

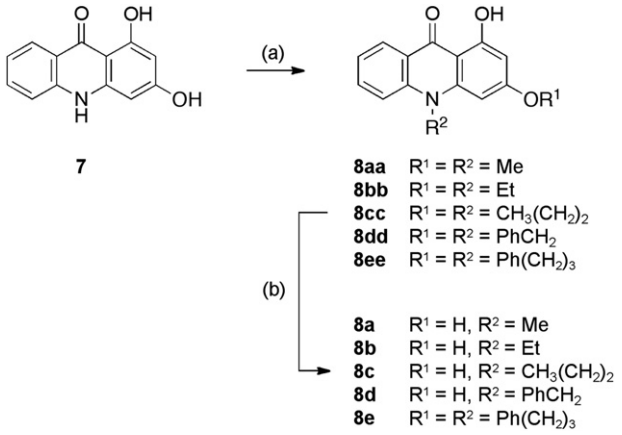
### 4.1. Inhibition of keratinocyte growth

In the antiproliferative study, each acridone derivative was tested for inhibitory action against HaCaT keratinocytes, as demonstrated by reduction in cell number over time as compared to control plates. The concentrations required to inhibit 50% of keratinocyte growth are shown in Table 1.

From the present work, 1,3-dihydroxy-acridone **8a** with an N-methyl substituent was identified as a highly potent inhibitor of keratinocyte proliferation, with an $IC_{50}$ value (0.6 μM) in the

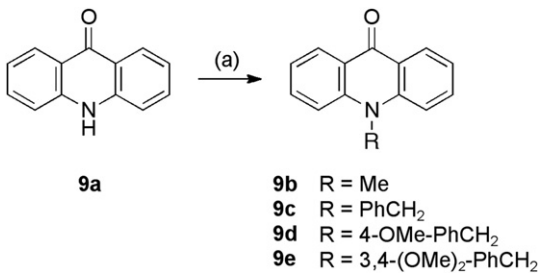



**Scheme 1.** Reagents: (a) alkyl halogenide, $K_2CO_3$, acetone, RT or reflux; (b) 48% HBr, reflux.

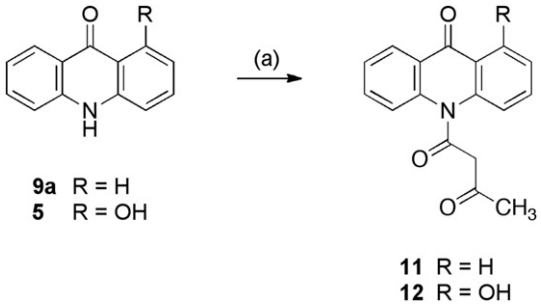



**Scheme 2.** Reagents: (a) alkyl halogenide, $K_2CO_3$, acetone, RT or reflux; (b) 48% HBr, reflux.

submicromolar range and comparable to that of standard anthralin. The naturally occurring alkaloid acronycine, which is also an N-methylated acridone and has shown activity in a large number of experimental tumor models [29], also displayed activity in the keratinocyte assay, with an $IC_{50}$ value in the low micromolar range. By contrast, unsubstituted acridone (**9a**) was not appreciably active, whereas the corresponding N-methylated **9b** displayed moderate activity. However, larger N-substituents as compared to **9b**, as in **9c**–**9e**, were detrimental for activity. In terms of SAR information, N-methylated acridone **9b** without hydroxy-substituent was substantially less potent than that hydroxylated at the 1,3-positions (**8a**), while compound **6a** with only one hydroxy-substituent at the 1-position was virtually inactive. As compared to the N-unsubstituted analogue **7**, potency of **8a** was dramatically improved by the 10-methyl substituent affording the most potent compound of all N-substituted acridones in this series. However, alkylation of the 3-hydroxy group (**8aa**, **8bb**, **8dd**) or only slight enlargements of the aliphatic 10-substituent as in N-ethylated **8b** or in N-propylated **8c** resulted in total loss of activity. In contrast to simple aliphatic substitution at the 10-position, compounds with an aliphatic chain terminated with a phenyl ring, in particular a benzyl substituent, were substantially more potent than their N-unsubstituted analogues. In the 1-hydroxy-substituted series (**6a**–**6l**), the benzylated analogues **6d** and **6e** as well as the methoxy-substituted analogues **6h** and **6i**, with an additional spacer group in the linker between the acridone scaffold and the phenyl ring, all displayed $IC_{50}$ values in the low micromolar range. On the other hand, further elongation of the linker chain as in **6k** and **6l** with three and four spacer groups, respectively, produced inactive compounds. In the 1,3-dihydroxy series (**8a**–**8e**), N-benzylated **8d** shared a comparable potency with the N-benzylated **6d** and **6e**. Of interest, also N-acylation of acridones **5** and **9a** gave active analogues (i.e., **11** and **12**), with analogue **12** being active in the low micromolar range.




**Scheme 3.** Reagents: (a) alkyl halogenide, $K_2CO_3$, acetone, reflux.




**Scheme 4.** Reagents: (a) NaH, THF, AcCl, RT, $N_2$.

There were four sets of compounds where the effect of N-methylation of the acridone scaffold could be evaluated, by comparing the corresponding NH and N-methyl derivatives. In two cases (i.e., **7**, **8a** and **9a**, **9b**), the N-methyl analogues were the more potent inhibitors of keratinocyte growth. In particular compound **8a** showed a 33-fold improvement over N-unsubstituted **7**. By contrast, the most potent inhibitor of our previous report, NH analogue **4**, completely lost its activity upon methylation (i.e., **10**). One pair of compounds (**5**, **6a**) could not be compared, as both were inactive.

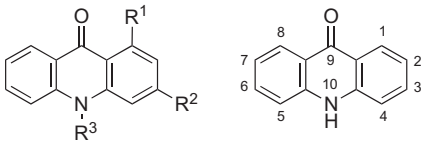
### 4.2. Membrane damage

The release of LDH is commonly used as an indicator of plasma-membrane damage. In this assay, LDH release by the standard anthralin, which was used as a positive control, significantly exceeded that of the vehicle control. On the other hand, the keratinocyte growth inhibitory activity of the naturally occurring acronycine and the most potent acridone derivatives was due to antiproliferative rather than non-specific cytotoxic effects, as LDH release was unchanged as compared to controls at 2-μM concentration of the test compounds (Table 1). The only exception was **6e**, which caused a slight increase in LDH activity as compared to vehicle controls. This may be related to its aromatic nitro group, even though direct radical production was excluded by the DPPH assay. However, enzymatic reduction of nitro aromatic compounds gives rise to nitro radical anions that may undergo redox cycling with oxygen to generate oxygen radicals [30]. Resultant oxidative stress can produce membrane damage.

### 4.3. Interaction with the stable free radical DPPH

Although scarcely described in the literature and only after photoirradiation and laser flash excitation of acridone, N-radical formation by hydrogen transfer to pyridine has been observed [31,32]. However, as N-substitution would prevent hydrogen transfer to a stable free radical such as DPPH, it is not surprising that with $EC_{50}$ values greater than 500 μM, none of the 10-substituted acridones showed any appreciable reactivity towards DPPH. These results were in excellent agreement with our data obtained from the LDH assay in which these novel compounds were devoid of membrane-damaging properties. As a matter of fact, this is beneficial as compared to anthralin, which was very effective in donating an electron to the stable free radical DPPH and, as a result, also caused desintegrity of keratinocyte membranes. The fact that the ability of the 10-substituted acridones to arrest keratinocyte growth was not mediated by non-specific redox properties or cytotoxic effects on the plasma-membrane, as observed for the antipsoriatic agent anthralin [15], opens new possibilities in the search for antipsoriatic agents.

**Table 1**
Antiproliferative activity against HaCaT keratinocytes, lactate dehydrogenase release, and free radical interacting capability of 10-substituted 10*H*-acridin-9-ones.




| cpd | Substitution pattern | AA[a] $IC_{50}$ (μM) | LDH[b] (mU/mL) | DPPH[d] $EC_{50}$ (μM) |
|---|---|---|---|---|
| **1** | Acronycine | 3.2 | 70.4 | >500 |
| **2** | Anthralin | 0.7 | 122.8[c] | 16.7 |
| **3**[f] | 1,8-$(OH)_2$ | 39.5 | [e] | >500 |
| **4**[f] | 1,3-$(OH)_2$8−Me | 0.8 | 71.5 | >500 |
| **5**[f,g] | $R^1$ = OH, $R^2$ = H, $R^3$ = H | >50 | [e] | >500 |
| **6a** | $R^1$ = OH, $R^2$ = H, $R^3$ = Me | >50 | [e] | [e] |
| **6aa** | $R^1$ = OMe, $R^2$ = H, $R^3$ = Me | >50 | [e] | [e] |
| **6b** | $R^1$ = OH, $R^2$ = H, $R^3$ = Et | >50 | [e] | [e] |
| **6c** | $R^1$ = OH, $R^2$ = H, $R^3$ = $CH_3(CH_2)_2$ | >50 | [e] | [e] |
| **6d** | $R^1$ = OH, $R^2$ = H, $R^3$ = $PhCH_2$ | 2.5 | 74.9 | >500 |
| **6dd** | $R^1$ = $OCH_2Ph$, $R^2$ = H, $R^3$ = $PhCH_2$ | 7.5 | 72.1 | >500 |
| **6ddd** | 1-OH-2,10-$(PhCH_2)_2$ | 13.0 | [e] | [e] |
| **6e** | $R^1$ = OH, $R^2$ = H, $R^3$ = 4-$NO_2$−$PhCH_2$ | 1.8 | 86.7[c] | >500 |
| **6f** | $R^1$ = OH, $R^2$ = H, $R^3$ = $Ph(CH_2)_2$ | 41.5 | [e] | [e] |
| **6g** | $R^1$ = OH, $R^2$ = H, $R^3$ = 4-OH−$Ph(CH_2)_2$ | 20.1 | [e] | >500 |
| **6h** | $R^1$ = OH, $R^2$ = H, $R^3$ = 4-OMe−$Ph(CH_2)_2$ | 6.3 | 74.6 | >500 |
| **6i** | $R^1$ = OH, $R^2$ = H, $R^3$ = 3,4-$(OMe)_2$−$Ph(CH_2)_2$ | 7.0 | [e] | >500 |
| **6j** | $R^1$ = OH, $R^2$ = H, $R^3$ = 3,4,5-$(OMe)_3$−$Ph(CH_2)_2$ | 45.5 | [e] | [e] |
| **6k** | $R^1$ = OH, $R^2$ = H, $R^3$ = $Ph(CH_2)_3$ | >50 | [e] | [e] |
| **6l** | $R^1$ = OH, $R^2$ = H, $R^3$ = $Ph(CH_2)_4$ | >50 | [e] | [e] |
| **7**[f,h] | $R^1$ = OH, $R^2$ = OH, $R^3$ = H | 19.8 | [e] | >500 |
| **8a** | $R^1$ = OH, $R^2$ = OH, $R^3$ = Me | 0.6 | 74.3 | >500 |
| **8aa** | $R^1$ = OH, $R^2$ = OMe, $R^3$ = Me | >50 | [e] | [e] |
| **8b** | $R^1$ = OH, $R^2$ = OH, $R^3$ = Et | >50 | [e] | [e] |
| **8bb** | $R^1$ = OH, $R^2$ = OEt, $R^3$ = Et | >50 | [e] | [e] |
| **8c** | $R^1$ = OH, $R^2$ = OH, $R^3$ = $CH_3(CH_2)_2$ | >50 | [e] | [e] |
| **8d** | $R^1$ = OH, $R^2$ = OH, $R^3$ = $PhCH_2$ | 3.7 | 76.8 | >500 |
| **8dd** | $R^1$ = OH, $R^2$ = $OPhCH_2$, $R^3$ = $PhCH_2$ | >50 | [e] | [e] |
| **8e** | $R^1$ = OH, $R^2$ = OH, $R^3$ = $Ph(CH_2)_3$ | 40.5 | [e] | [e] |
| **9a** | $R^1$ = $R^2$ = $R^3$ = H | >50 | [e] | [e] |
| **9b**[i] | $R^1$ = $R^2$ = H, $R^3$ = Me | 38.9 | [e] | [e] |
| **9c**[i] | $R^1$ = $R^2$ = H, $R^3$ = $PhCH_2$ | >50 | [e] | [e] |
| **9d**[k] | $R^1$ = $R^2$ = H, $R^3$ = 4-OMe−$PhCH_2$ | 46.3 | [e] | >500 |
| **9e** | $R^1$ = $R^2$ = H, $R^3$ = 3,4-$(OMe)_2$−$PhCH_2$ | >50 | [e] | >500 |
| **10** | 1,3-$(OH)_2$-8,10-$Me_2$ | >50 | [e] | [e] |
| **11** | $R^1$ = $R^2$ = H, $R^3$ = $COCH_2COMe$ | 27.8 | [e] | [e] |
| **12** | $R^1$ = OH, $R^2$ = H, $R^3$ = $COCH_2COMe$ | 3.3 | 74.0 | >500 |

[a] Antiproliferative activity against keratinocytes. $IC_{50}$, concentration of test compound required for 50% inhibition of cell growth (HaCaT). Inhibition of cell growth was significantly different with respect to that of the control, $N = 3$, $P < 0.05$.
[b] Activity of LDH (mU) release in HaCaT cells after treatment with 2 μM test compound ($N = 3$, SD < 10%).
[c] Values are significantly different with respect to vehicle control (0.2% DMSO in the culture medium, 70.6 mU/mL), $P < 0.05$. Brij 35 (polyoxyethyleneglycol dodecyl ether)/ultrasound was the positive control (251 mU/mL).
[d] DPPH radical interacting capability. $EC_{50}$, effective concentration of test compound ($N = 3$, SD < 10%) required for 50% decrease in DPPH absorbance at 520 nm. NDGA ($IC_{50}$ = 4.2 μM) and α-tocopherol ($IC_{50}$ = 8.2 μM) were used as positive controls.
[e] Not determined.
[f] Ref. [23].
[g] Ref. [36].
[h] Ref. [33].
[i] Ref. [34].
[k] Ref. [8].

## 5. Conclusions

The aim of this study was to elucidate the SAR of 10-substituted acridone analogues related to the antipsoriatic agent anthralin, to further delineate the number and position of hydroxy and other groups at the acridone scaffold that contribute to the antiproliferative activity. As already observed in our previous series of N-unsubstituted acridones [23], a 1,3-dihydroxy-substitution pattern was most beneficial for potency. One member of this series, acridone **8a**, with an N-methyl substituent at the 1,3-dihydroxy-substituted skeleton, was detected as a highly potent inhibitor of keratinocyte growth. With an $IC_{50}$ value of 0.6 μM, potency of the anthrone derivative anthralin was retained. As already seen with the most potent analogue of our previous series, acridone **4**, which is an isomer of **8a**, this N-methylated acridone was not capable of interacting with DPPH and thus producing radicals that give rise to a non-specific cytotoxic action, which would preclude a possible use in dermatology. This was in excellent agreement with the fact that in contrast to anthralin, the most potent acridones of this series did not disrupt membrane integrity. Also of interest, acylation of the acridone nitrogen atom resulted in active compounds such as **12**, whose potency may further be improved with an appropriate substitution pattern. In conclusion,

among our series of acridone analogues we were able to identify potent inhibitors of human keratinocyte growth at sufficiently low concentrations to warrant investigation as antipsoriatic agents in more sophisticated systems.

## 6. Experimental section

### 6.1. Chemistry

Melting points were determined with a Kofler melting point apparatus and are uncorrected. $^1$H NMR and $^{13}$C NMR spectra were recorded with a Varian Gemini 200 (200 and 50.29 MHz, respectively) spectrometer, using tetramethylsilane as an internal standard. Fourier-transform IR spectra (KBr) were recorded on a Bio-Rad laboratories type FTS 135 spectrometer. Mass spectra were obtained in the EI mode using a MAT GCQ Finnigan instrument. Thin layer chromatography (TLC) was conducted on Merck 60 $F_{254}$ precoated silica gel plates. Chromatography refers to column chromatography, which was performed on Merck silica gel (70–230 mesh). Yields have not been optimized. Elemental analyses were determined by the Microanalysis Laboratory at the University of Münster using a Vario EL III elemental analyzer. Elemental analyses were within ±0.4% of calculated values, except where stated otherwise.

Acridones **6a**, **6aa** [25], **8a**, **8aa** [33], **9b**, **9c** [34] **9d** [8], and **10** [35] were prepared according to literature methods. Acridone **9a** was commercially available (Sigma–Aldrich).

#### 6.1.1. General procedure for alkylation of hydroxyacridones

*6.1.1.1. 10-Ethyl-1-hydroxy-10H-acridin-9-one (**6b**).* A mixture of 1-hydroxyacridone [36] (**5**, 0.20 g, 0.95 mmol), dry, finely mortared $K_2CO_3$ (0.41 g, 3 mmol) and iodoethane (3.11 g, 19.95 mmol) in dry acetone (30 mL) was stirred under reflux for 5 h. Then the mixture was filtered by suction, the residue dissolved in $CH_2Cl_2$ and filtered. The combined filtrate was evaporated and the residue was purified by chromatography ($SiO_2$; $CH_2Cl_2$/MeOH, 99/1) to provide yellow crystals; 40% yield; mp 161 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 14.59 (s, 1H, OH), 8.34 (d, $J$ = 7.7 Hz, 1H, H-8), 7.90–7.34 (m, 4H, H-3, H-5, H-6, H-7), 7.19 (d, $J$ = 8.7 Hz, 1H, H-4), 6.64 (d, $J$ = 8.1 Hz, 1H, H-2), 4.53 (q, $J$ = 7.2 Hz, 2H, $CH_2CH_3$), 1.38 (t, $J$ = 7.1 Hz, 3H, $CH_2CH_3$); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 181.3, 162.8, 142.0, 141.1, 120.0, 109.2, 136.6, 135.2, 126.0, 121.7, 115.8, 106.7, 104.4, 41.1, 34.7; FTIR 1630 cm$^{-1}$ (CO); MS $m/z$ = 239 (100, M$^+$). Anal. $C_{15}H_{13}NO_2$ (C, H, N).

*6.1.1.2. 1-Hydroxy-10-propyl-10H-acridin-9-one (**6c**).* The title compound was obtained from **5** and 1-iodopropane using the same procedure as for **6b**, but the mixture was refluxed for 24 h. Purification by chromatography ($SiO_2$; $CH_2Cl_2$) gave golden yellow crystals; 63% yield; mp 92 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 14.59 (s, 1H), 8.32 (d, $J$ = 7.6 Hz, 1H), 7.87–7.32 (m, 4H), 7.15 (d, $J$ = 8.9 Hz, 1H), 6.62 (d, $J$ = 8.0 Hz, 1H), 4.37 (t, $J$ = 8.1 Hz, 2H), 1.83–1.72 (m, 2H), 1.05 (t, $J$ = 7.3 Hz, 3H); FTIR 1634 cm$^{-1}$ (CO); MS $m/z$ = 253 (100, M$^+$). Anal. $C_{16}H_{15}NO_2$ (C, H, N).

*6.1.1.3. 10-Benzyl-1-hydroxy-10H-acridin-9-one (**6d**).* The title compound was obtained from **5** and benzyl bromide using the same procedure as for **6b**, but the mixture was refluxed for 24 h. Purification by chromatography ($SiO_2$; $CH_2Cl_2$) gave gold-colored crystals; 77% yield; mp 176 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 14.50 (s, 1H, OH), 8.30 (d, $J$ = 7.3 Hz, 1H, H-8), 7.89–7.12 (m, 9H, Ar), 6.95 (d, $J$ = 8.8 Hz, 1H, H-4), 6.65 (d, $J$ = 8.0 Hz, 1H, H-2), 5.79 (s, 2H, $CH_2$); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 181.5, 162.7, 142.9, 142.0, 136.5, 135.8, 135.1, 128.7 (2C), 127.2, 125.8, 125.7 (2C), 122.0, 120.1, 116.1, 109.1, 107.1, 104.9, 49.6; FTIR 1630 cm$^{-1}$ (CO); MS $m/z$ = 301 (70, M$^+$), 91 (100). Anal. $C_{20}H_{15}NO_2$ (C, H, N).

*6.1.1.4. 10-Benzyl-1-benzyloxy-10H-acridin-9-one (**6dd**).* The title compound was obtained as a side product of **6d**. Purification by chromatography ($SiO_2$; $CH_2Cl_2$) gave gold-colored crystals; 5% yield; mp 179 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 8.32 (d, $J$ = 6.7 Hz, 1H, H-8), 7.73–7.13 (m, 14H, Ar), 7.05 (d, $J$ = 8.7 Hz, 1H, H-4), 6.91 (d, $J$ = 8.1 Hz, 1H, H-2), 5.68 (s, 2H, $OCH_2$), 5.26 (s, 2H, $NCH_2$); FTIR 1632 cm$^{-1}$ (CO); MS $m/z$ = 391 (100, M$^+$). Anal. $C_{27}H_{21}NO_2$ (C, H, N).

*6.1.1.5. 2,10-Dibenzyl-1-hydroxy-10H-acridin-9-one (**6ddd**).* The title compound was obtained as a side product of **6d**, when the mixture was refluxed for 50 h. Purification by chromatography ($SiO_2$; $CH_2Cl_2$) gave yellow crystals; 13% yield; mp 191 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 14.91 (s, 1H, OH), 8.39 (dd, $J$ = 8.1, 1.7 Hz, 1H, H-8), 7.83–7.10 (m, 14H, Ar), 6.96 (d, $J$ = 8.9 Hz, 1H, H-4), 5.74 (s, 2H, $NCH_2$Ar), 3.94 (s, 2H, Ar$CH_2$Ar); FTIR 1622 cm$^{-1}$ (CO); MS $m/z$ = 391 (100, M$^+$). Anal. $C_{27}H_{21}NO_2$ (C, H, N).

*6.1.1.6. 1-Hydroxy-10-(4-nitrobenzyl)-10H-acridin-9-one (**6e**).* The title compound was obtained from **5** and 4-nitrobenzyl bromide using the same procedure as for **6b**, but the mixture was refluxed for 9 h. Purification by chromatography ($SiO_2$; $CH_2Cl_2$) gave yellow crystals; 46% yield; mp 282 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 14.59 (s, 1H, OH), 8.40 (dd, $J$ = 1.4, 8.1 Hz, 1H, H-8), 8.18 (d, $J$ = 8.5 Hz, 2H, H-3′, H-5′), 7.82 (t, $J$ = 7.3 Hz, 1H, H-6), 7.66–7.39 (m, 3H, H-3, H-5, H-7), 7.41 (d, $J$ = 8.5 Hz, 2H, H-2′, H-6′), 6.96 (d, $J$ = 8.7 Hz, 1H, H-4), 6.68 (d, $J$ = 8.0 Hz, 1H, H-2), 5.94 (s, 2H, $CH_2$); FTIR 1638 cm$^{-1}$ (CO); MS $m/z$ = 346 (100, M$^+$). Anal. $C_{20}H_{14}N_2O_4$ (C, H, N).

*6.1.1.7. 1-Hydroxy-10-(2-phenylethyl)-10H-acridin-9-one (**6f**).* The title compound was obtained from **5** and 2-bromoethylbenzene using the same procedure as for **6b**, but the mixture was refluxed for 24 h. Purification by chromatography ($SiO_2$; $CH_2Cl_2$/MeOH, 98/2) gave yellow crystals; 12% yield; mp 162 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 14.59 (s, 1H, OH), 8.36 (d, $J$ = 7.9 Hz, 1H, H-8), 7.89–7.20 (m, 10H, Ar), 6.66 (d, $J$ = 7.8 Hz, 1H, H-2), 4.66 (t, $J$ = 8.0 Hz, 2H, $NCH_2CH_2$), 3.10 (t, $J$ = 8.1 Hz, 2H, $NCH_2CH_2$); FTIR 1630 cm$^{-1}$ (CO); MS $m/z$ = 315 (91, M$^+$), 224 (100). Anal. $C_{21}H_{17}NO_2$ (C, H, N).

*6.1.1.8. 1-Hydroxy-10-[2-(4-hydroxyphenyl)ethyl]-10H-acridin-9-one (**6g**).* The title compound was obtained from **6h** using the same procedure as for **8b**. Purification by chromatography ($SiO_2$; $CH_2Cl_2$) gave yellow crystals; 43% yield; mp 230 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 14.59 (s, 1H), 9.28 (s, br, 1H), 8.35 (d, $J$ = 7.7 Hz, 1H), 7.89–6.64 (m, 10H), 4.58 (t, $J$ = 8.0 Hz, 2H), 2.98 (t, $J$ = 8.0 Hz, 2H); FTIR 3329, 1637 cm$^{-1}$ (CO); MS $m/z$ = 331 (48, M$^+$), 212 (100). Anal. $C_{21}H_{17}NO_3$ (C, H, N).

*6.1.1.9. 1-Hydroxy-10-[2-(4-methoxyphenyl)ethyl]-10H-acridin-9-one (**6h**).* The title compound was obtained from **5** and 1-(2-bromoethyl)-4-methoxybenzene [37] using the same procedure as for **6b**, but the mixture was refluxed for 24 h. Purification by chromatography ($SiO_2$; twice: hexane, then $CH_2Cl_2$) gave yellow crystals; 24% yield; mp 126 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 14.59 (s, 1H), 8.35 (d, $J$ = 7.8 Hz, 1H), 7.88 (d, $J$ = 3.1 Hz, 1H), 7.71 (t, $J$ = 8.3 Hz, 1H), 7.39–7.33 (m, 2H), 7.35 (d, $J$ = 8.6 Hz, 2H), 7.21 (d, $J$ = 8.5 Hz, 1H), 6.88 (d, $J$ = 8.4 Hz, 2H), 6.66 (d, $J$ = 8.0 Hz, 1H), 4.60 (t, $J$ = 8.1 Hz, 2H), 3.72 (s, 3H), 3.03 (t, $J$ = 7.9 Hz, 2H); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 181.3, 162.8, 158.1, 142.1, 141.2, 136.6, 135.2, 130.0 (2C), 129.5, 125.9, 121.8, 120.0, 115.9, 113.9 (2C), 109.0, 106.9, 104.6, 55.0, 47.4, 31.6; FTIR 1633 cm$^{-1}$ (CO); MS $m/z$ = 345 (20, M$^+$), 134 (100). Anal. $C_{22}H_{19}NO_3$ (C, H, N).

*6.1.1.10. 1-Hydroxy-10-[2-(3,4-dimethoxyphenyl)ethyl]-10H-acridin-9-one (**6i**).* The title compound was obtained from **5** and 1-(2-bromoethyl)-3,4-dimethoxybenzene [38] using the same procedure as

for **6b**, but the mixture was refluxed for 24 h. Purification by chromatography ($SiO_2$; twice: $CH_2Cl_2$, then $CH_2Cl_2$/MeOH, 98/2) gave yellow-orange crystals; 28% yield; mp 125 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 14.59 (s, 1H), 8.35 (d, $J$ = 7.7 Hz, 1H), 7.89–6.64 (m, 9H), 4.64 (t, $J$ = 7.8 Hz, 2H), 3.74 (s, 3H), 3.70 (s, 3H), 3.02 (t, $J$ = 7.9 Hz, 2H); FTIR 1635 cm$^{-1}$ (CO); MS $m/z$ = 375 (17, M$^+$), 164 (100). Anal. $C_{23}H_{21}NO_4$ (C, H, N).

*6.1.1.11. 1-Hydroxy-10-[2-(3,4,5-trimethoxyphenyl)ethyl]-10H-acridin-9-one (**6j**).* The title compound was obtained from **5** and 1-(2-bromoethyl)-3,4,5-trimethoxybenzene [38] using the same procedure as for **6b**, but the mixture was refluxed for 24 h. Purification by chromatography ($SiO_2$; twice: $CH_2Cl_2$, then $CH_2Cl_2$/MeOH, 98/2) gave yellow-orange crystals; 5% yield; mp 156 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 14.60 (s, 1H), 8.35 (d, $J$ = 7.9 Hz, 1H), 7.88–6.61 (m, 8H), 4.71 (t, $J$ = 7.5 Hz, 2H), 3.72 (s, 6H), 3.58 (s, 3H), 3.02 (t, $J$ = 7.6 Hz, 2H); FTIR 1630 cm$^{-1}$ (CO); MS $m/z$ = 405 (19, M$^+$), 194 (100). Anal. $C_{24}H_{23}NO_5$ (C, H, N).

*6.1.1.12. 1-Hydroxy-10-(3-phenylpropyl)-10H-acridin-9-one (**6k**).* The title compound was obtained from **5** and 3-bromopropylbenzene using the same procedure as for **6b**, but the mixture was refluxed for 24 h. Purification by chromatography ($SiO_2$; $CH_2Cl_2$) gave yellow crystals; 55% yield; mp 120 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 14.56 (s, 1H, OH), 8.31 (dd, $J$ = 1.7, 8.1 Hz, 1H, H-8), 7.82–7.20 (m, 9H, Ar), 6.99 (d, $J$ = 8.6 Hz, 1H, H-4), 6.60 (d, $J$ = 7.9 Hz, 1H, H-2), 4.39 (t, $J$ = 8.0 Hz, 2H, NC$H_2$CH$_2$CH$_2$), 2.84 (t, $J$ = 7.7 Hz, 2H, NCH$_2$CH$_2$C$H_2$), 2.08–2.01 (m, 2H, NCH$_2$C$H_2$CH$_2$); FTIR 1620 cm$^{-1}$ (CO); MS $m/z$ = 329 (69, M$^+$), 224 (100). Anal. $C_{22}H_{19}NO_2$ (C, H, N).

*6.1.1.13. 1-Hydroxy-10-(4-phenylbutyl)-10H-acridin-9-one (**6l**).* The title compound was obtained from **5** and 4-bromobutylbenzene [39] using the same procedure as for **6b**, but the mixture was refluxed for 24 h. Purification by chromatography ($SiO_2$; hexane/$CH_2Cl_2$, 85/15) gave yellow crystals; 90% yield; mp 108 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 14.58 (s, 1H), 8.31 (dd, $J$ = 1.5, 8.0 Hz, 1H), 7.83–7.15 (m, 9H), 7.07 (d, $J$ = 8.7 Hz, 1H), 6.61 (d, $J$ = 7.9 Hz, 1H), 4.42 (t, $J$ = 8.3 Hz, 2H), 2.66 (t, $J$ = 6.8 Hz, 2H), 1.80–1.76 (m, 4H); FTIR 1628 cm$^{-1}$ (CO); MS $m/z$ = 343 (100, M$^+$). Anal. $C_{23}H_{21}NO_2$ (C, H, N).

*6.1.1.14. 3-Ethoxy-10-ethyl-1-hydroxy-10H-acridin-9-one (**8bb**).* The title compound was obtained from 1,3-dihydroxyacridone (**7**) [33] and iodoethane using the same procedure as for **6b**, but the mixture was refluxed for 24 h. Purification by chromatography ($SiO_2$; $CH_2Cl_2$/MeOH, 98/2) gave yellow crystals; 68% yield; mp 170–172 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 14.92 (s, 1H, OH), 8.26 (d, $J$ = 8.2 Hz, 1H, H-8), 7.81–7.78 (m, 2H, H-5, H-6), 7.31 (dd, $J$ = 8.0, 5.8, 2.1 Hz, 1H, H-7), 6.47 (d, $J$ = 2.1 Hz, 1H, H-4), 6.22 (d, $J$ = 1.9 Hz, 1H, H-2), 4.42 (q, $J$ = 7.0 Hz, 2H, OC$H_2$CH$_3$), 4.15 (q, $J$ = 6.9 Hz, 2H, NC$H_2$CH$_3$), 1.39–1.30 (m, 6H, OCH$_2$C$H_3$, NCH$_2$C$H_3$); FTIR 1639 cm$^{-1}$ (CO); MS $m/z$ = 283 (100, M$^+$). Anal. $C_{17}H_{17}NO_3$ (C, H, N).

*6.1.1.15. 10-Benzyl-3-benzyloxy-1-hydroxy-10H-acridin-9-one (**8dd**).* The title compound was obtained from **7** and benzyl bromide using the same procedure as for **6b**, but the mixture was stirred at RT for 24 h. Purification by chromatography ($SiO_2$; hexane/$CH_2Cl_2$, 1/1) and recrystallization from $CH_2Cl_2$/MeOH (1/1) gave pale-yellow crystals; 20% yield; mp 224–225 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 14.66 (s, 1H), 8.17 (d, $J$ = 8.1 Hz, 1H), 7.86–7.10 (m, 14H), 6.48 (s, 1H), 5.21 (s, 2H), 4.30 (s, 2H); FTIR 3345, 1635 cm$^{-1}$ (CO); MS $m/z$ = 407 (75, M$^+$), 316 (100). Anal. $C_{27}H_{21}NO_3$ (C, H, N).

*6.1.1.16. 10-(3,4-Dimethoxybenzyl)-10H-acridin-9-one (**9e**).* The title compound was obtained from acridone (**9a**) and 3,4-dimethoxybenzyl bromide [40] using the same procedure as for **6b**, but the mixture was refluxed for 24 h. Purification by chromatography ($SiO_2$; $CH_2Cl_2$) gave orange crystals; 20% yield; mp 215–216 °C; $^1$H NMR ($CDCl_3$) $\delta$ 8.60 (dd, $J$ = 8.0 Hz, $J$ = 1.8 Hz, 2H, H-1, H-8), 7.76–7.54 (m, 2H, H-3, H-6), 7.46–7.16 (m, 4H, H-2, H-4, H-5, H-7), 7.02–6.74 (m, 2H, H-2′, H-5′), 6.46–6.28 (m, 1H, H-6′), 5.63 (s, 2H, NC$H_2$), 4.07 (s, 3H, OCH$_3$), 3.94 (s, 3H, OCH$_3$); FTIR 3345, 1634 cm$^{-1}$ (CO); MS $m/z$ = 345 (38, M$^+$), 151 (100). Anal. $C_{22}H_{19}NO_3$ (C, H, N).

*6.1.2. General procedure for the cleavage of ethers*

*6.1.2.1. 10-Ethyl-1,3-dihydroxy-10H-acridin-9-one (**8b**).* A solution of **8bb** (0.62 g, 2.20 mmol) in 48% HBr (30 mL) was refluxed for 3 h. Then the solution was allowed to cool to RT. The precipitating crystals were filtered by suction, washed with water (2 × 100 mL), dissolved in $CH_2Cl_2$ (100 mL), and the aqueous phase was extracted with $CH_2Cl_2$ (2 × 100 mL). The combined organic phase was dried over $Na_2SO_4$, evaporated, and the residue purified by chromatography ($SiO_2$; $CH_2Cl_2$/MeOH, 98/2) to provide yellow-green crystals; 94% yield; mp 293 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 14.90 (s, 1H, 1-OH), 10.65 (s, 1H, 3-OH), 8.28 (d, $J$ = 7.4 Hz, 1H, H-8), 7.85–7.19 (m, 3H, H-5, H-6, H-7), 6.43 (d, $J$ = 1.7 Hz, 1H, H-4), 6.11 (d, $J$ = 1.7 Hz, 1H, H-2), 4.37 (q, $J$ = 6.9 Hz, 2H, C$H_2$CH$_3$), 1.36 (t, $J$ = 7.0 Hz, 3H, CH$_2$C$H_3$); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 179.9, 165.0, 164.9, 143.6, 140.9, 120.0, 103.7, 134.4, 125.8, 121.3, 115.3, 95.9, 90.8, 41.0, 11.9; FTIR 1655 cm$^{-1}$ (CO); MS $m/z$ = 255 (100, M$^+$). Anal. $C_{15}H_{13}NO_3$ (C, H, N).

*6.1.2.2. 1,3-Dihydroxy-10-propyl-10H-acridin-9-one (**8c**).* The title compound was obtained from **7** [33] and 1-iodopropane using the same procedure as for **6b**, but the mixture was refluxed for 24 h. The crude product **8cc** was then processed as described for **8b**. Purification by chromatography ($SiO_2$; $CH_2Cl_2$/MeOH, 98/2) gave yellow crystals; 29% yield; mp 213 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 14.89 (s, 1H), 10.65 (s, 1H), 8.25 (d, $J$ = 8.2 Hz, 1H), 7.83–7.71 (m, 2H), 7.30 (t, $J$ = 7.0 Hz, 1H), 6.38 (d, $J$ = 1.7 Hz, 1H), 6.09 (d, $J$ = 1.6 Hz, 1H), 4.20 (t, $J$ = 7.8 Hz, 2H), 1.81–1.69 (m, 2H), 1.05 (t, $J$ = 7.3 Hz, 3H); FTIR 1639 cm$^{-1}$ (CO); MS $m/z$ = 269 (82, M$^+$), 227 (100). Anal. $C_{16}H_{15}NO_3$ (C, H, N).

*6.1.2.3. 10-Benzyl-1,3-dihydroxy-10H-acridin-9-one (**8d**).* The title compound was obtained from **8dd** as described for **8b**, but the mixture was refluxed for 9 h. Purification by chromatography ($SiO_2$; $CH_2Cl_2$) gave yellow crystals; 13% yield; mp 233 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 14.50 (s, 1H), 10.69 (s, br, 1H), 8.14 (dd, $J$ = 8.1, 1.2 Hz, 1H), 7.82–7.07 (m, 9H), 6.19 (s, 1H), 4.21 (s, 2H); FTIR 3402, 1650 cm$^{-1}$ (CO); MS $m/z$ = 317 (100, M$^+$). Anal. $C_{20}H_{15}NO_2$ (C, H, N).

*6.1.2.4. 1,3-Dihydroxy-10-(3-phenylpropyl)-10H-acridin-9-one (**8e**).* The title compound was obtained from **7** [33] and 3-bromopropylbenzene using the same procedure as for **6b**, but the mixture was refluxed for 48 h. The crude product **8ee** was then processed as described for **8b**. Purification by chromatography ($SiO_2$; $CH_2Cl_2$/MeOH, 99/1) gave yellow crystals; 22% yield; mp 204 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 14.89 (s, 1H), 10.69 (s, 1H), 8.26 (dd, $J$ = 8.0, 1.3 Hz, 1H), 7.75 (t, $J$ = 7.2 Hz, 1H), 7.60 (d, $J$ = 8.7 Hz, 1H), 7.34–7.17 (m, 6H), 6.36 (d, $J$ = 1.3 Hz, 1H), 6.10 (d, $J$ = 1.4 Hz, 1H), 4.27 (t, $J$ = 7.2 Hz, 2H), 2.84 (t, $J$ = 7.6 Hz, 2H), 2.07–2.03 (m, 2H); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 179.3, 165.0 (2C), 143.9, 141.1, 141.0, 134.4, 128.4 (2C), 128.2 (2C), 126.0, 125.7, 121.4, 119.9, 115.2, 103.7, 95.9, 90.6, 45.6, 31.9, 27.9; FTIR 1638 cm$^{-1}$ (CO); MS $m/z$ = 345 (99, M$^+$), 241 (100). Anal. $C_{22}H_{19}NO_3$ (C, H, N).

*6.1.3. 1-(9-Oxo-9H-acridin-10-yl)butane-1,3-dione (**11**)*

To a solution of acridone (**9a**, 1.0 g, 5.1 mmol) in dimethylformamide (70 mL) was added NaH (60%, 0.19 g) under $N_2$. The mixture was stirred at RT for 1 h. Then acetyl chloride (2 mL, 28.0 mmol) was added. Three further successive additions of NaH

followed by acetyl chloride were made over 48 h. The mixture was poured into ice-water (120 mL) and the residue filtered by suction. Purification by chromatography ($SiO_2$; $CH_2Cl_2$) gave pale-yellow crystals; 26% yield; mp 121 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 8.34–7.36 (m, 8H), 5.84 (d, $J$ = 2.6 Hz, 2H), 2.15 (s, 3H); FTIR 1764 (N–CO), 1608 cm$^{-1}$ (CO); MS $m/z$ = 279 (55, M$^+$), 195 (100). Anal. $C_{17}H_{13}NO_3$ (C, H, N).

#### 6.1.4. 1-(1-Hydroxy-9-oxo-9H-acridin-10-yl)butane-1,3-dione (**12**)

The title compound was obtained from **5** [36] (0.5 g, 2.4 mmol) in THF (40 mL) as described for **11**. The reaction mixture was treated with water (80 mL) and extracted with $CH_2Cl_2$ (3 × 30 mL). The combined organic phase was dried over $Na_2SO_4$, evaporated, and the resulting red-brown oil was purified by chromatography ($SiO_2$; $CH_2Cl_2$/MeOH, 99/1) to afford yellow crystals; 12% yield; mp 149 °C; $^1$H NMR ($CD_3OD$) $\delta$ 13.98 (s, 1H, OH), 8.34–6.51 (m, 7H, Ar), 5.82 (d, $J$ = 2.6 Hz, 2H, $CH_2$), 2.16 (s, 3H, $CH_3$); FTIR 1774 (N–CO), 1637 cm$^{-1}$ (CO); MS $m/z$ = 295 (49, M$^+$), 211 (100). Anal. $C_{17}H_{13}NO_4$ (C, H, N).

### 6.2. Biological assay methods

#### 6.2.1. Keratinocyte culture and determination of cell growth

HaCaT cells [26] were cultivated and the cell proliferation assay was performed as described [41]. 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; $P < 0.05$). $IC_{50}$ values were obtained by nonlinear regression.

#### 6.2.2. Lactate dehydrogenase release

The assay was performed as described [19,27]. 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 ($P < 0.05$; $N$ = 3, SD < 10%). Brij 35 (polyoxyethyleneglycol dodecyl ether)/ultrasound was the positive control.

#### 6.2.3. Determination of DPPH radical interacting capability

The assay was performed as described [23]. The capability to interact with DPPH radical (%) was calculated by the comparison of the mean values of the test compound ($N$ = 3) with those of the control ($N$ = 8): (1−absorbance of test compound/absorbance of control) × 100. Results were expressed as $EC_{50}$, the effective concentration of the test compound required for 50% decrease in DPPH absorbance at 520 nm. Values were obtained by nonlinear regression.

## Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2010.08.059.

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