Alkoxypsoralens, Novel Nonpeptide Blockers of Shaker-Type K⁺ Channels: Synthesis and Photoreactivity

Heike Wulff,* Heiko Rauer,† Tim Durig,‡ Christine Hanselmann, Katharina Ruff, Anja Wrisch, Stephan Grissmer, and Wolfram Hänsel*

Pharmaceutical Institute and Physiological Institute, University of Kiel, Gutenbergstr. 76, 24118 Kiel, Germany

Received May 5, 1998

A series of psoralens and structurally related 5,7-disubstituted coumarins was synthesized and investigated for their K⁺ channel blocking activity as well as for their phototoxicity to Artemia salina and their ability to generate singlet oxygen and to photomodify DNA. After screening the compounds on Ranvier nodes of the toad Xenopus laevis, the affinities of the most promising compounds, which proved to be psoralens bearing alkoxy substituents in the 5-position or alkoxymethyl substituents in the neighboring 4- or 4′-position, to a number of homomeric K⁺ channels were characterized. All compounds exhibited the highest affinity to Kv1.2. 5,8-Diethoxypsoralen (10d) was found to be an equally potent inhibitor of Kv1.2 and Kv1.3, while lacking the phototoxicity normally inherent in psoralens. The reported compounds represent a novel series of nonpeptide blockers of Shaker-type K⁺ channels that could be further developed into selective inhibitors of Kv1.2 or Kv1.3.

Introduction

Voltage-gated K⁺ channels play a cardinal role in the regulation of physiological functions in excitable as well as nonexcitable cells. In demyelinating diseases such as multiple sclerosis (MS), destruction of the myelin sheath evokes an internodal delayed rectifier K⁺ current by uncovering normally silent K⁺ channels. These abnormal K⁺ currents contribute to the conduction failure observed in MS by changing the axonal resting potential. Blockers of axonal K⁺ channels such as 4-aminopyridine (4-AP), which has been demonstrated to be able to overcome conduction failure in experimentally demyelinated nerve fibers, are therefore considered useful for the symptomatic treatment of multiple sclerosis and other demyelinating diseases such as diabetic neuropathy. 4-AP, which is currently undergoing phase III clinical trials, has been shown to reduce disability in certain MS patients. It is known that paranodal K⁺ channels in mammals are probably heteromultimers encoded by the Shaker-related genes Kv1.1 and Kv1.2. Selective blockers of Kv1.1 and Kv1.2 are accordingly supposed to be potential therapeutic agents for the treatment of demyelinating diseases. As demyelination in MS is mediated by autoreactive T-cells, another validated target for the treatment of multiple sclerosis should be Kv1.3, a Shaker-related K⁺ channel involved in control of membrane potential, production of lymphokines, and proliferation of human T-lymphocytes. 4-AP, tetrathyamineon chloride (TEA), quinine, and verapamil block Kv1.3 and in parallel potency sequence inhibit T-lymphocyte activation. Studies with the more potent and selective peptide antagonists charybotoxin, margatoxin (MgTX), and kaliotoxin have corroborated these findings. Recently MgTX has been shown to suppress delayed-type hypersensitivity and allogenic-antibody responses in miniswine, providing in vivo evidence that Kv1.3 is a novel pharmacological target for immunosuppressive therapy.

In voltage clamp experiments on amphibian nodes of Ranvier, 5-methoxypsoralen (5-MOP) was found to selectively block delayed rectifier K⁺ currents. Moreover, single trials have shown that 5-MOP can alleviate functional deficits in certain MS patients. Psoralens such as 5- and 8-MOP are widely used in PUVA (psoralen plus ultraviolet-A radiation) therapy of psoriasis, vitiligo, and mycosis fungoides and, more recently, for cutaneous T-cell lymphoma by means of photopheresis, an extracorporeal form of photothermotherapy. Psoralens are planar bifunctional photocross-linkable agents that are capable of intercalating into double-stranded DNA. Upon irradiation with UVA, the 3,4- or 4′,5′-double bond of the pyrone or furan groups can undergo a [2+2] cycloaddition reaction with the 5,6-dibond of thymine residues forming monoadducts. If the psoralen has intercalated into a suitable site, monoadducts can undergo a further cycloaddition with the other strand of the DNA duplex to produce an interstrand cross-link. This photodamage to DNA is responsible for the antiproliferative, photomutagenic, and photocarcinogenic effects of psoralens. However, it has been shown that psoralens can also photomodify biomolecules other than DNA, i.e., proteins and lipids, either by direct photoaddition or by generation of singlet oxygen, suggesting cell membranes to be one of the major targets of PUVA therapy. Since the phototoxic and photomutagenic activity of 5-MOP might pose serious problems for its use in the long-term therapy of demyelinating disease, the aim of this study was to synthesize new psoralen derivatives in order to separate their K⁺ channel blocking activity from their inherent phototoxicity.
The synthesis of 5,7-dialkoxycoumarins is illustrated in Scheme 1. 1a was prepared from phloroglucinol according to the method of Kaufman and Kelly. Pechmann reaction of phloroglucinol and methyl 4-methoxyacetoacetate gave 2a. 1a and 2a were treated with the appropriate alkyl halides to provide 1b, c and 2b.

3 was obtained from 3,4-(methylendioxy)phenol according to the method of Woods. Synthesis of 4′-substituted psoralens (Scheme 2) was achieved in two steps by modifying the method of MacLeod. 7-Hydroxycoumarins were converted to the β-keto ethers 4a-9a by reaction with chloroacetone or α-chloroacetophenone. Treatment of these compounds in refluxing 1 M aqueous KOH under nitrogen for 24 h followed by acidification afforded the psoralen derivatives 4b-9b. The 1H NMR spectra confirmed the exclusive cyclization to the linear furocoumarin skeleton; the corresponding angular furocoumarins were not detectable.

10d was prepared via Scheme 3. Demethylation of 8-MOP with magnesium iodide yielded 10a, which on oxidation with chromium trioxide gave the quinone 10b. Reduction of the quinone with zinc dust in dilute HCl provided the hydroquinone 10c, which was reacted with diethyl sulfate in the presence of potassium carbonate to give target molecule 10d.

The 4′,5′-dihydropsoralen derivatives 11–13 were obtained by transfer hydrogenation of the necessary psoralens according to the method of Heindel using cyclohexene in refluxing ethanol as a hydrogen donor under catalysis by Pd/C (Scheme 4). Nitration of 8-MOP gave 14a, and subsequent reduction gave 14b.

Chloromethylation of 5-MOP, 8-MOP, and trioxsalen followed by nucleophilic substitution of the resulting chloromethylpsoralens 15a–17a with the appropriate alcohols or amines is illustrated in Scheme 5.

**Results and Discussion**

**Blockade of Axonal K⁺ Currents.** In order to rapidly assess the K⁺ channel blocking activity of the compounds, they were screened on Ranvier nodes of the toad *Xenopus laevis*. This approach is based on the assumption that the electrophysiological and pharmacological properties of uncovered internodal potassium channels in mammals are close to those described for amphibian nodes of Ranvier. By investigating the single-channel properties of the delayed rectifier K⁺ channels (I-type) in peripheral myelinated axons from *X. laevis*, Koh and Vogel have found these channels to show many similarities to Kv1.1; the slight differences probably reflect the fact that the channels from *Xenopus* are heteromultimers of Kv1.1 and another Kv1 R-subunit. The screening technology has been described previously. Briefly, the blockade of steady-state...
potassium currents in the test solution, $B_K$, at pulse amplitudes of 130 mV was normalized to the corresponding $K^+$ currents in normal bathing medium. The accompanying blockade of peak $Na^+$ currents, measured at pulse amplitudes of 70 mV, was defined in the same way, so that the selectivity $S$ of the respective $K^+$ current blockade is given by $B_K/B_{Na}$. The results of the screening are presented in Table 1.

Whereas hydrophilic 5,7-substituted coumarins (1a, c, 2a, and 3) were found to be completely inert at 100 $\mu$M, their much more lipophilic 5,7-diethoxy ethers (1b and 2b) proved to be nonselective blockers of both axonal $Na^+$ and $K^+$ currents. The $K^+$ channel blocking potency of the tested psoralens is dependent on the substitution pattern. Whereas psoralens bearing an alkyl substituent in the 8-position (8-MOP and 16b–e) show only a weak blocking function, 5-alkoxypsoralens such as 5-MOP, 15b, and 10d were found to be potent blockers of axonal $K^+$ currents while leaving axonal $Na^+$ currents largely unaffected. Psoralens bearing alkoxymethyl substituents in the neighboring 4- and 4′-positions such as 4b and 17b,c are equally effective. Hydrogenation of the furan ring does not significantly alter the efficacy of the compounds (12 and 13). Bulk and hydrophobicity of substituents in the 4′-position appear to be an important feature for determining the selectivity of the tested psoralens. Whereas 4′-phenyl-substituted psoralens such as 5b and 8b suppress both axonal $Na^+$ and $K^+$ currents, psoralens bearing the much smaller methyl group in the 4′-position are highly selective (4b and 6b).

Similar to the investigated coumarins, the incorporation of polar substituents into the psoralen system or into a side chain such as hydroxy (9b and 10a,c), keto (10b), nitro (14a), or amino (14b) groups greatly diminishes or completely abolishes activity. The quaternary ammonium compounds 16f and 17d, which combine the psoralen moiety and TEA in one molecule, showed activity only at 0.5 mM. Instead of binding to the hydrophobic binding site, we postulate for uncharged, lipophilic psoralens such as 5-MOP (log P = 2.08) and 10d (log P = 2.88), quaternized psoralens apparently act on the external TEA binding site of $K^+$ channels.

Taken together, the data on this set of compounds suggest that psoralens possess an internal hydrophobic binding site, which membrane-impermeable compounds such as 16f and 17d cannot reach and to which psoralens bearing polar substituents lack affinity. Optimal substituents seem to be alkoxy groups in the 5-position or alkoxymethyl groups in the neighboring 4- and 4′-positions.

### Effects on Homomeric $K^+$ Channels

Of the psoralens screened on Ranvier nodes, we further investigated the most promising compounds (5-MOP, 4b, and 10d) for their affinities to different homomeric $K^+$ channels of the Shaker family and the Shaw family and also to the SK Ca channel in Jurkat T-cells. Kv1.1, Kv1.2, Kv1.3, Kv1.5, Kv1.6, and Kv3.1 channels were exogenously expressed either by transfection or by injection in mammalian cells.35,36 The expressed channels were electrophysiologically characterized in the whole-cell configuration using the patch-clamp technique. Each compound was tested at different concentrations. Values for the apparent dissociation constant, $K_d$, of blocking peak $K^+$ currents were calculated by fitting a modified Hill equation to the data points. The $K_d$ values are listed in Table 2.

### Table 1. Compound Structure and Block of Steady-State Axonal $K^+$ Currents

<table>
<thead>
<tr>
<th>compd</th>
<th>$R_1$</th>
<th>$R_2$</th>
<th>$R_3$</th>
<th>$B_K$ at 25 $\mu$M (%)</th>
<th>$S$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1b</td>
<td>H</td>
<td></td>
<td></td>
<td>40</td>
<td>0.7</td>
</tr>
<tr>
<td>2b</td>
<td>CH$_3$OCH$_3$</td>
<td>C$_2$H$_5$</td>
<td>35</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>3b</td>
<td></td>
<td></td>
<td></td>
<td>52</td>
<td>14</td>
</tr>
<tr>
<td>4b</td>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>5b</td>
<td></td>
<td></td>
<td>CH$_3$</td>
<td>52</td>
<td>17</td>
</tr>
<tr>
<td>6b</td>
<td></td>
<td></td>
<td>CH$_3$</td>
<td>27$^a$</td>
<td>2</td>
</tr>
<tr>
<td>7b</td>
<td></td>
<td></td>
<td></td>
<td>63</td>
<td>15</td>
</tr>
<tr>
<td>8b</td>
<td></td>
<td></td>
<td></td>
<td>25</td>
<td>8</td>
</tr>
<tr>
<td>9b</td>
<td></td>
<td></td>
<td></td>
<td>68$^a$</td>
<td>1.4</td>
</tr>
<tr>
<td>10d</td>
<td></td>
<td></td>
<td></td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
<td>CH$_3$</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td>23</td>
<td>3</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td>45</td>
<td>22</td>
</tr>
<tr>
<td>15b</td>
<td></td>
<td></td>
<td></td>
<td>35</td>
<td>26</td>
</tr>
<tr>
<td>16b</td>
<td></td>
<td></td>
<td>OCH$_3$</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td>16c</td>
<td></td>
<td></td>
<td>OCH$_3$</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>16d</td>
<td></td>
<td></td>
<td>OCH(CH$_3$)$_2$</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>16e</td>
<td></td>
<td></td>
<td>OCH-CH=CH$_2$</td>
<td>24</td>
<td>6</td>
</tr>
<tr>
<td>16f</td>
<td>N$^+$</td>
<td></td>
<td>C$_2$H$_5$</td>
<td>20$^f$</td>
<td>7</td>
</tr>
<tr>
<td>17b</td>
<td></td>
<td></td>
<td>OCH$_3$</td>
<td>31</td>
<td>11</td>
</tr>
<tr>
<td>17c</td>
<td></td>
<td></td>
<td>OCH$_3$</td>
<td>55</td>
<td>6</td>
</tr>
<tr>
<td>17d</td>
<td>N$^+$</td>
<td></td>
<td>C$_2$H$_5$</td>
<td>27$^f$</td>
<td>5</td>
</tr>
</tbody>
</table>

$^a$ $B_K$, blockade of steady-state $K^+$ currents normalized to the corresponding $K^+$ currents in normal bathing medium (pulse amplitude 130 mV, pulse duration 100 ms); $B_{Na}$, accompanying blockade of peak $Na^+$ currents (pulse amplitude 70 mV); $S$, selectivity of $K^+$ current blockade given by $B_K/B_{Na}$. $^b$ Tested at 10 $\mu$M. $^c$ Tested at 0.5 mM.
Alkoxypsoralens, Nonpeptide Blockers of $K^+$ Channels

Table 2. Sensitivity of $K^+$ Channels of the Shaker and Shaw Families to Blocka

<table>
<thead>
<tr>
<th>K$_a$ ($\mu$M)</th>
<th>Kvl1</th>
<th>Kvl2</th>
<th>Kvl3</th>
<th>Kvl5</th>
<th>Kvl6</th>
<th>Kvl6</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-AP$^{35}$</td>
<td>290</td>
<td>590</td>
<td>195</td>
<td>270</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>5-MOP$^{15b}$</td>
<td>&gt;200</td>
<td>41</td>
<td>102</td>
<td>177</td>
<td>187</td>
<td>111</td>
</tr>
<tr>
<td>4b</td>
<td>56</td>
<td>18</td>
<td>90</td>
<td>nd</td>
<td>nd</td>
<td>213</td>
</tr>
<tr>
<td>10d</td>
<td>167</td>
<td>23</td>
<td>28</td>
<td>nd</td>
<td>nd</td>
<td>178</td>
</tr>
<tr>
<td>15b</td>
<td>nd</td>
<td>nd</td>
<td>28</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

a K$_a$ dissociation constant of blockade of peak $K^+$ currents (mean of at least three experiments); nd, not determined.

Table 3. Photobinding to Poly(dA-dT)-Poly(dA-dT)-DNA Determined by the Shift of Thermal Transition Temperature and Production of Singlet Oxygen

<table>
<thead>
<tr>
<th>compd</th>
<th>LC$_{50}$ (M)</th>
<th>photoinduced dose modification</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-MOP</td>
<td>&gt;100</td>
<td>2.5</td>
</tr>
<tr>
<td>8-MOP</td>
<td>&gt;100</td>
<td>2.5</td>
</tr>
<tr>
<td>5,8-dimOP</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>4b</td>
<td>&gt;100</td>
<td>2.5</td>
</tr>
<tr>
<td>6b</td>
<td>&gt;100</td>
<td>0.5</td>
</tr>
<tr>
<td>7b</td>
<td>&gt;100</td>
<td>0.7</td>
</tr>
<tr>
<td>10d</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>12</td>
<td>&gt;100</td>
<td>25</td>
</tr>
<tr>
<td>13</td>
<td>&gt;100</td>
<td>25</td>
</tr>
<tr>
<td>15b</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>16b</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>17b</td>
<td>&gt;100</td>
<td>20</td>
</tr>
</tbody>
</table>

Mean value (±0.5 °C) from three determinations for a [compound][DNA] ratio of 1:1. *Decrease in absorbance of N,N-dimethyl-p-nitrosoaniline (RNO) at 440 nm after 5 h of UV-A irradiation (4.5 mW/cm$^2$; incident UV-A dose of 81 J/cm$^2$).

The phototoxic properties of the compounds were determined against the nauplii of the marine crustacean Artemia salina, the brine shrimp. By the name of Artoxkit M, it is one of the “cyst-based toxicity tests” commonly used in ecotoxicology.$^{41}$ As A. salina has recently been described to be extremely sensitive to toxicity mediated by active oxygen species such as superoxide and $1^O_2$, the standard test procedure was modified by additional UV-A irradiation in order to use this test to determine the photoinduced toxicity of the synthesized psoralens. The LC$_{50}$ values are given in Table 4.

Under daylight none of the compounds were found to be toxic up to 100 μM, which is consistent with the LC$_{50}$ values of 340 μM described for 5-MOP, 8-MOP, and 5,8-dimethoxypsoralen on HeLa cells.$^{43}$ Following UV-A irradiation a number of compounds showed an increase in toxicity, which was particularly striking for 6b and 7b which exhibited photoinduced dose modifications of >200 and >150, respectively. The “irradiated” LC$_{50}$ values found for 5- and 8-MOP (2.5 μM) were again in agreement with those described for HeLa and A 431 cells (2.5–4.3 μM).$^{44,45}$ 5,8-Dimethoxypsoralen, 10d, 15b, and 16b were completely devoid of acute phototoxicity to A. salina.

Conclusions

The synthesis and pharmacological testing of a series of psoralens and structurally related 5,7-disubstituted coumarins as blockers of voltage-gated $K^+$ channels have been described. The compounds were also investigated for their phototoxicity to A. salina, their ability to generate singlet oxygen, and their ability to photomodify DNA. By synthesizing 10d and 15b we succeeded in separating the $K^+$ channel blocking activity in the presence of the sensitizer histidine (0.01 M). The decrease in absorbance during UV-A irradiation, recorded at 440 nm, is shown in Table 3. 4'-Methylpsoralens (4b and 6b), dihydropсорalens (12), and derivatives of trioxsalen (17b,c) were found to be effective producers of $1^O_2$. As expected the reference compounds 5- and 8-MOP were only weak producers of $1^O_2$; their derivatives 15b and 16c also exhibited low activity. 5,8-Dialkoxypsoralens such as 5,8-dimethoxypsoralen (isosiprinellin) and 10d were found to completely lack the ability to generate $1^O_2$; the negative decrease in absorbance noted is probably due to the fact that these compounds are weak antioxidants, which prevent the slow “self-bleaching” of RNO observed under UV-A irradiation.

Photobinding to DNA. Since thymine is by far the preferred pyrimidine base in psoralen photobinding, the amount of drug covalently bound to a respective polynucleotide generally increases with its A-T content. Repeated A-T sequences are described to be “hot-spots” for psoralen cross-linking.$^{37}$ We therefore used poly(dA-dT)-poly(dA-dT) to study the photoactivity of the compounds to DNA.

Photobinding of psoralens stabilizes the helix leading to an increase in thermal helix → coil transition temperature ($T_m$).$^{38}$ $T_m$ increases determined by recording the melting profiles of poly(dA-dT)-poly(dA-dT) in the presence and absence of drug are summarized in Table 3. Apart from the quaternary compound 17d, which meets all the requirements for a classical intercalator (a planar chromophore and a flexible basic side chain), none of the compounds had any marked effect on $T_m$ in the dark. After 30 min of irradiation 4b and 17b,c had apparently formed cross-links with the polynucleotide to such an extent that no real melting curve could be observed any more. The reference compounds 5- and 8-MOP induced a shift of $T_m$ of 13 and 10 °C, respectively. 5,8-Dimethoxypsoralen, 10d, 15b, and 16c exhibited no effect on $T_m$ despite irradiation, a finding suggesting that these compounds do not produce any photoadducts with DNA.

Formation of Singlet Oxygen. The generation of $1^O_2$ by various compounds (10 μM) was determined in $O_2$-saturated solutions according to the method of Krägel and El Mohsni,$^{39}$ which is based on the bleaching of N,N-dimethyl-p-nitrosoaniline (50 μM) by $1^O_2$ in the presence of the sensitizer histidine (0.01 M). The decrease in absorbance during UV-A irradiation, recorded at 440 nm, is shown in Table 3.
of psoralens from their phototoxic and photomutagenic properties. Its nearly equivalent affinity to Kv1.2 and Kv1.3 makes \textit{10d} a potential therapeutic agent, that in addition to its symptomatic effects on impulse propagation in demyelinated axons could simultaneously modify the immune response in multiple sclerosis by suppressing the activity of autoreactive T-cells. Quite recently \textit{10d} has actually been shown to inhibit the proliferation and interferon-γ mRNA expression of lymph node cells of Lewis rats with experimental autoimmune encephalomyelitis, the animal model of MS.\textsuperscript{30} Although the substances might still lack the high selectivity and potency required of a therapeutic drug, they could serve as templates for the design of either Kv1.2- or Kv1.3-selective K⁺ channel blockers.

**Experimental Section**

Melting points were determined with a Reichert Thermovar apparatus and are uncorrected. Preparative column chromatography was performed using silica gel 60 (0.063–0.100 mm; Merck). Infrared (IR) spectra were recorded on a Nicolet-505/2100 or 1600 series FTIR (KBr disks, cm⁻¹). Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker ARX 300 spectrometer, and chemical shifts (ppm) are reported relative to the solvent peak (CDCl₃ or DMSO-d₆ at 7.24 ppm and DMSO in DMSO-d₆ at 2.49 ppm) or TMS. Signals are designated as follows: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet), ex (exchangeable with D₂O). \textsuperscript{1}H NMR peaks were assigned with the help of COLOC, COSY, and NOESY experiments. Complete NMR data are only given for the first compound of each series; for the others only relevant resonances are given. Mass spectra were run on a Hewlett-Packard 5989A spectrometer (EI, 70 eV). Elemental analyses were performed by a Hewlett-Packard CHN autoanalyzer and were within ±0.4% of the theoretical values. Yields were not optimized. The \textsuperscript{1}H NMR and MS spectra of known compounds were in agreement with their chemical structure.

7-Hydroxy-4-(methoxymethyl)coumarin was purchased from Maybridge. Compounds \textit{3, 9b, 10a, 10b, 11a, 14b, 15a, 16a, 17a, 16f, 16g, 17a, 17b, 11b, 17b, 18a, 28} were synthesized as described previously. Compounds \textit{6a, 6f, 7a, 19a} and \textit{29} were prepared according to general method A; compounds \textit{6b, 6g, 7b, 19b} and \textit{28b} were prepared according to general method B.

### 5,7-Dihydroxycoumarin (1a)

To a solution of phloroglucinol anhydrous (11.8 g, 0.12 mol) in dioxane (100 mL) were added ethyl propiolate (11.8 g, 0.12 mol) and freshly dried zinc chloride (10.8 g).\textsuperscript{31} After stirring under reflux for 24 h, the mixture was poured into HCl (5%, 100 mL), concentrated chloride (10.8 g). After stirring under reflux for 24 h the mixture was poured into HCl (5%, 100 mL), concentrated (10.8 g). After stirring under reflux for 24 h the mixture was poured into HCl (5%, 100 mL), concentrated (10.8 g). After stirring under reflux for 24 h the mixture was poured into HCl (5%, 100 mL), concentrated (10.8 g). After stirring under reflux for 24 h the mixture was poured into HCl (5%, 100 mL), concentrated (10.8 g). After stirring under reflux for 24 h the mixture was poured into HCl (5%, 100 mL), concentrated (10.8 g). After stirring under reflux for 24 h the mixture was poured into HCl (5%, 100 mL), concentrated (10.8 g). After stirring under reflux for 24 h the mixture was poured into HCl (5%, 100 mL), concentrated (10.8 g). After stirring under reflux for 24 h the mixture was poured into HCl (5%, 100 mL), concentrated (10.8 g). After stirring under reflux for 24 h the mixture was poured into HCl (5%, 100 mL), concentrated (10.8 g). After stirring under reflux for 24 h the mixture was poured into HCl (5%, 100 mL), concentrated (10.8 g). After stirring under reflux for 24 h the mixture was poured into HCl (5%, 100 mL), concentrated (10.8 g). After stirring under reflux for 24 h the mixture was poured into HCl (5%, 100 mL), concentrated (10.8 g). After stirring under reflux for 24 h the mixture was poured into HCl (5%, 100 mL), concentrated (10.8 g). After stirring under reflux for 24 h the mixture was poured into HCl (5%, 100 mL), concentrated (10.8 g). After stirring under reflux for 24 h the mixture was poured into HCl (5%, 100 mL), concentrated (10.8 g). After stirring under reflux for 24 h the mixture was poured into HCl (5%, 100 mL), concentrated (10.8 g).

### 5,7-Dihydroxycoumarin (1b)

To a solution of 1a (1.0 g, 5.6 mmol) in anhydrous acetonitrile (100 mL) were added potassium carbonate (10 g) and chloroacetone (0.90 mmol). After heating to reflux for 2 h the mixture was poured into HCl (300 mL), acidified with HCl, and kept at 4 °C overnight. The precipitate formed was collected by vacuum filtration and recrystallized from methanol (modified after MacLeod).\textsuperscript{32}

### 5,7-Bis(2-Oxopropoxy)coumarin (1c)

To a solution of 1b (617 mg, 74%) of 7-(2-Oxopropoxy)-5,7-dihydroxycoumarin was made using phloroglucinol anhydrous (100 mL) and chloroacetone (1.8 mL, 20.0 mmol). After heating to reflux under stirring for 6 h, the mixture was poured into cold water (300 mL) and kept at 4 °C overnight. The precipitate formed was collected by vacuum filtration and recrystallized from methanol (modified after MacLeod).\textsuperscript{32}

### 5,7-Phenylpsoralens

#### General Method A: Preparation of 7-(2-Oxopropoxy)- and 7-(Phenacyloxy)coumarins.

To a solution of \textit{7-Hydroxycoumarins (20.0 mmol)} in anhydrous acetone (100 mL) were added potassium carbonate (10 g) and chloroacetone (1.8 mL, 20.0 mmol). After heating to reflux under stirring for 6 h, the mixture was poured into cold water (300 mL), acidified with HCl, and kept at 4 °C overnight. The precipitate formed was collected by vacuum filtration and recrystallized from methanol (modified after MacLeod).\textsuperscript{32}

#### General Method B: Preparation of 4-Methyl- and 4-Phenylpsoralens

To a solution of \textit{7-(2-Oxopropoxy)- and 7-(Phenacyloxy)coumarins (17b, 11b)} was prepared from \textit{4-(Methoxymethyl)-7-(2-Oxopropoxy)coumarin (4a)}.

### 7-(Methoxymethyl)coumarin (2b)

To a solution of \textit{3, 4b} (0.50 g, 2.3 mmol) according to the method given for \textit{1b} (617 mg, 74%) of 7-(2-Oxopropoxy)-5,7-dihydroxycoumarin (11.8 g, 0.12 mol) in anhydrous acetone (100 mL) were added potassium carbonate (10 g) and chloroacetone (1.8 mL, 20.0 mmol). After heating to reflux under stirring for 6 h, the mixture was poured into cold water (300 mL), acidified with HCl, and kept at 4 °C overnight. The precipitate formed was collected by vacuum filtration and recrystallized from methanol (modified after MacLeod).\textsuperscript{32}

### 5,7-Bis(2-Oxopropoxy)coumarin (1c)

To a solution of 1b was prepared from \textit{3, 4b} (0.50 g, 2.3 mmol) according to the method given for \textit{1b} (617 mg, 74%) of 7-(2-Oxopropoxy)-5,7-dihydroxycoumarin (11.8 g, 0.12 mol) in anhydrous acetone (100 mL) and chloroacetone (1.8 mL, 20.0 mmol). After heating to reflux under stirring for 6 h, the mixture was poured into cold water (300 mL), acidified with HCl, and kept at 4 °C overnight. The precipitate formed was collected by vacuum filtration and recrystallized from methanol (modified after MacLeod).\textsuperscript{32}

### 5,7-Bis(2-Oxopropoxy)coumarin (1c)

To a solution of \textit{4b} (1,4 g, 7.8 mmol) and chloroacetone (0.90 mmol, 11b) was prepared from \textit{4-(Methoxymethyl)-7-(2-Oxopropoxy)coumarin (4a)}.

### 4-(Methoxymethyl)-7-(2-Oxopropoxy)coumarin (4a)

To a solution of \textit{4a} (1 g, 5.8 mmol) according to the method B (1.0 g, 5.8 mmol) was suspended in 1 M potassium hydroxide solution (100 mL) and heated to reflux under nitrogen for 24 h. After cooling the solution was acidified with phosphoric acid and kept at 4 °C overnight. The precipitate formed was collected by vacuum filtration and recrystallized from methanol (modified after MacLeod).\textsuperscript{32}
were concentrated and dried to give 3:1 ethyl acetate/cyclohexane as eluent. The desired fractions residue, which was purified by column chromatography using (3 mmol) of 5a, HCl was added dropwise. After 5 h the remain-

ion and recrystallized from water (311 mg, 62%): mp 268 °C and 3-go of zinc dust were suspended in a mixture of acetone (217 mg, 0.72 mmol) was heated under reflux in 50 mL of methanol according to the method of Hansen58 formed in 2-mL quartz cuvettes using a HP 8845A diode-array coil transition

(200 mg, 0.72 mmol) was heated under reflux in 50 mL of methanol according to the method of Hansen58 formed in 2-mL quartz cuvettes using a HP 8845A diode-array coil transition

8-(Chloromethyl)-5-methoxypsoralen (15a). Compound 15a was prepared from 5-MOP (0.50 g, 2.3 mmol) according to the method of Iisaac36 given for the chlorimination of trioxalen (407 mg, 67%): mp 200 °C; 1H NMR (300 MHz, CDCl3) δ 8.16 (d, 1H, J = 9.8 Hz, 4-H), 7.64 (d, 1H, J = 2.2 Hz, 5-H), 7.04 (d, 1H, J = 2.2 Hz, 4-H), 6.30 (d, 1H, J = 9.8 Hz, 3-H), 5.07 (s, 2H, 8-CH2Cl), 4.29 (s, 3H, 5-OCH3); MS m/z 264 (M+, 43), 229 (100), 201 (19), 186 (24).

5-Methoxy-8-(methylthioperyl)xpsoralen (15b). 15a (400 mg, 1.5 mmol) was heated under reflux in 100 mL of methanol for 6 h. The solvent was evaporated and the crude product recrystallized from methanol to give 15b (246 mg, 63%): mp 176 °C; 1H NMR (300 MHz, CDCl3) δ 4.91 (s, 2H, 8-CH2-OC6H5), 4.26 (s, 3H, 5-OCH3), 3.38 (s, 3H, 8-CH3-OC6H5); 13C NMR (75 MHz, CDCl3) δ 160.80 (C-2), 157.74 (C-7), 150.80 (C-8a), 149.49 (C-14), 149.90 (C-5), 133.93 (C-4), 112.27 (C-3), 106.18 (C-6), 105.51 (C-5), 105.15 (C-4), 104.73 (C-8), 62.18 (8-CH3-OC6H5), 60.03 (5-OCH3), 58.48 (8-CH2-OC6H5); MS m/z 266 (M+, 39), 229 (100), 201 (23), 186 (24), 173 (6), 158 (5). Anal. (C19H2O4S) C, H.

4-(Ethoxymethyl)-4,5,8-trimethylpsoralen (17c). 17a (200 mg, 0.72 mmol) was heated under reflux in 50 mL of ethanol for 6 h. The solvent was evaporated and the crude product recrystallized from ethanol to give 17c (160 mg, 82%): mp 124 °C; 1H NMR (300 MHz, CDCl3) δ 7.61 (s, 2H, 5-H), 6.24 (s, 1H, 4-H), 4.62 (s, 2H, 4-CH2-OC6H5), 3.55 (q, 2H, 4-CH2-OC6H5), 2.57-2.49 (3 s, 9H, 4′-CH3, 5′-CH3, and 8-CH3), 1.25 (t, 3H, 4′-CH2-OC6H5); 13C NMR (75 MHz, CDCl3) δ 161.54 (C-2), 155.05 (C-5′), 154.71 (C-7), 153.36 (C-6), 149.21 (C-8a), 125.10 (C-4′), 116.14 (C-4), 112.74 (C-3), 112.16 (C-4a), 111.61 (C-5), 110.01 (C-8), 65.47 (4′-CH2-OC6H5-"CH3, 62.79 (4′-CH2-OC6H5-"CH3, 19.35 (5′-CH3), 15.24 (4′-CH2-OC6H5), 12.33 (4′-CH3), 8.47 (8-CH3); MS m/z 286 (M+, 35), 257 (5), 241 (62), 227 (17), 213 (16), 212 (284), 199 (20), 185 (8), 141 (16), 128 (27), 115 (28), 106 (17). Anal. (C18H20O5S) C, H.

4-(Triethylammoniomethyl)-4,5,8-trimethylpsoralen Chloride (17d). Compound 17d was prepared from 17a (200 mg, 0.72 mmol) according to the method of Hansen58 given for 16f (247 mg, 91%): mp 214 °C; 1H NMR (300 MHz, DMSO) δ 7.02 (s, 2H, 5-H), 6.40 (s, 1H, 4-H), 4.80 (s, 2H, 4′-CH2-N(CH3)2, 3.33 (m, 6H, 4′-CH2-N(CH3)2, 2.75-2.50 (3 s, 9H, 4′-CH3, 5′-CH3, 8-CH3), 1.34 (t, 9H, 4′-CH2-N(CH3)2, 13C NMR (75 MHz, DMSO) δ 159.83 (C-3), 124.89 (C-4), 104.57 (4′-CH2-N(CH3)2, 52.83 (4′-CH2-N(CH3)2, 18.81 (5′-CH3), 13.25 (4′-CH2-N(CH3)2), 9.71 (8-CH3); MS (thermospray, LC/MS) m/z 378 (MH+), 342 (29), 249 (10), 277 (40), 258 (39), 241 (86), 102 (100). Anal. (C21H28O6NCl) C, H, Cl.

UV-A Irradiation. Irradiations were performed by means of a 150-W Heraeus Fluotest mercury-vapor lamp; irradiation intensities, determined with a Holtek UV-A meter 360, were 4.5 and 1.3 mW/cm2 at a distance of 20 and 45 cm, respectively.

Cytosolic and Membrane Transition Studies. Melting studies were performed in 2-mL quartz cuvettes using a HP 8845A diode-array spectrophotometer fitted with a J ulabo F20-C heating controller. The temperature was directly measured in the cuvettes with a digital thermometer. Heating was applied at a rate of 1 °C min-1, with absorbance (260 nm) and temperature data sampling at 1-min intervals. DNA helix - coil transition temperatures (Tm) were determined at the midpoint of the
meting profiles. Drug-induced alterations in the DNA melting behavior are given by: $\Delta T_m = T_m(\text{DNA} + \text{drug}) - T_m(\text{DNA})$, where the $T_m$ for the drug-free DNA control is $34.0 \pm 0.2^\circ$C; results are given as the mean from three determinations.

A stock solution containing 1 unit of the polynucleotide in 100 $\mu$L was prepared by dissolving 10 units of poly(dA-dT)-poly(dA-dT)-DNA (Sigma P-0883) in 1 mL of 2-(N-morpholino)-propanesulfonic acid (MOPS) buffer (0.1 M, pH 7.0); working solutions contained 10 $\mu$L M DNA (20 $\mu$L of the stock solution). DNA–drug solutions were prepared by addition of the compounds from a stock solution in methanol (10 $M$) to give a final drug concentration of 10 $M$. The solutions were either incubated for 30 min in the dark or irradiated for 30 min at 365 nm with 4.5 mW/cm$^2$ (incident UV-A dose of 8.1 J/cm$^2$). The addition of 20 $\mu$L of methanol and UV-A irradiation for 30 min had no effect on the transition temperature of the polynucleotide in absence of drug.

**In Vitro Phototoxicity to *A. salina***. Brine shrimp (Artemia salina) cysts were hatched in a flask of real seawater at 25°C, with continuous side illumination and with aeration maintained by a commercial aquarium pump. After 48 h the phototactic nauplii were collected using a Pasteur pipet. Ten nauplii were transferred to each sample vial containing 3 mL of seawater and 100 nm, 500 nm, 1 $M$, 2.5 $M$, 5 $M$, 10 $M$, 25 $M$, 50 $M$, or 100 $M$ of the compounds, which were added from methanolic stock solutions. Every vial contained 2% (v/v) methanol; it was separately established that this level of methanol was well-tolerated. The nauplii were incubated at 25°C with the test compounds either under daylight or with additional UV-A irradiation (1.3 mW/cm$^2$, incident UV-A dose of 112 J/cm$^2$). After 24 h the number of dead nauplii was noted in each vial. All assays were performed in triplicate. Under the conditions employed UV-A exposure was completely non-toxic in the absence of drug.

**Acknowledgment.** The authors wish to thank Prof. Dr. E. Koppenhöfer for making the screening experiments possible and Prof. Dr. D. Heber for helpful discussions throughout this work. Financial support from the DFG (Gr848/4-1 and Gr848/4-2) and from the BMBF (IZKF Ulm, Projekt B1) is gratefully acknowledged.

**References**


Alkoxypsoralens, Nonpeptide Blockers of K⁺ Channels


