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

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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.[1,2] 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.[3] These abnormal K^+ currents contribute to the conduction failure observed in MS by changing the axonal resting potential.[4] 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,[5] 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.[6,7] It is known that paranodal K^+ channels in mammals are probably heteromultimers encoded by the *Shaker*-related genes Kv1.1 and Kv1.2.[8] 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,[9] 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.[10,11] 4-AP, tetraethylammonium chloride (TEA), quinine, and verapamil block Kv1.3 and in parallel potency sequence inhibit T-lymphocyte activation.[10−13] Studies with the more potent and selective peptide antagonists charybdotoxin, margatoxin (MgTX), and kaliotoxin have corroborated these findings.[14,15] Recently MgTX has been shown to suppress delayed-type hypersensitivity and allogenic-antibody responses in miniswine,[16] providing in vivo evidence that Kv1.3 is a novel pharmacological target for immunosuppressive therapy.[17,18]

In voltage clamp experiments on amphibian nodes of Ranvier, 5-methoxypsoralen (5-MOP) was found to selectively block delayed rectifier K^+ currents.[19] Moreover, single trials have shown that 5-MOP can alleviate functional deficits in certain MS patients.[20]

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 photochemotherapy.[21] Psoralens are planar bifunctional photoactivatable agents that are capable of intercalating into double-stranded DNA. Upon irradiation with UV-A, 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-double bond 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.[22] This photodamage to DNA is responsible for the antiproliferative, photomutagenic, and photocarcinogenic effects of psoralens.[23] However, it has been shown that psoralens can also photomodify biomacromolecules other than DNA,[24] 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.[25] Since the phototoxic and photomutagenic activity of 5-MOP[26] 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.

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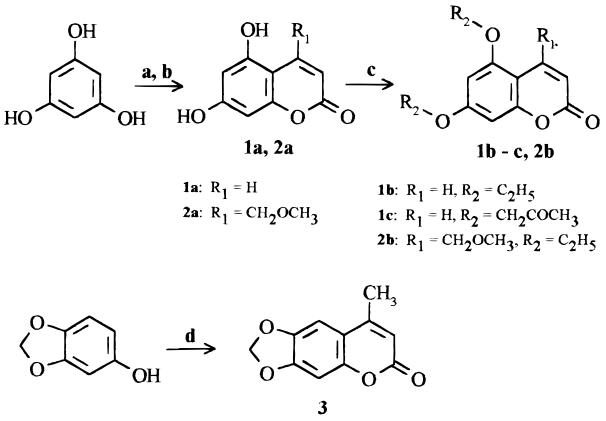
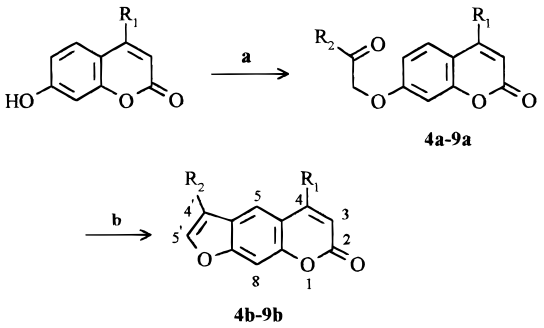
10.1021/jm981032o CCC: $15.00
Published on Web 10/08/1998

Scheme 1. Synthetic Route to Alkoxycoumarins[a]



[a] Reagents: (a) ethyl propiolate, $ZnCl_2$, dioxane; (b) methyl 4-methoxyacetoacetate, H_2SO_4; (c) alkyl halide, acetone, K_2CO_3; (d) ethyl acetoacetate, H_2SO_4.

Scheme 2. Synthetic Route to 4′-Substituted Psoralens[a]



[a] Reagents: (a) $ClCH_2COR_2$, acetone, K_2CO_3; (b) 1 M KOH, N_2.

Chemistry

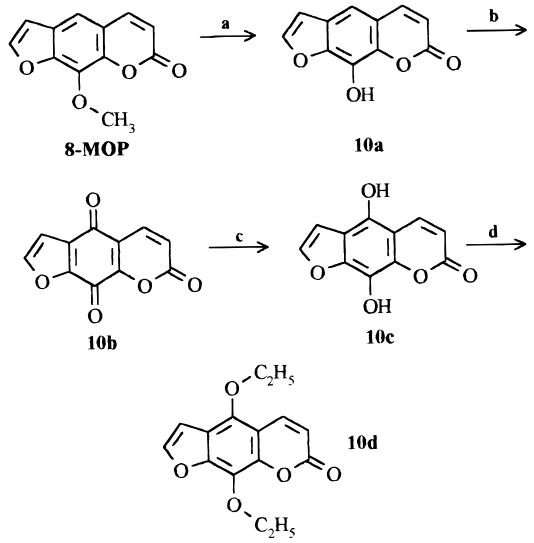
The synthesis of 5,7-dialkoxycoumarins is illustrated in Scheme 1. **1a** was prepared from phloroglucinol according to the method of Kaufman and Kelly.[27] Pechmann reaction of phloroglucinol and methyl 4-methoxyacetoacetate gave **2a**. **1a** and **2a** were treated with the appropriate alkyl halides to provide **1b**,**c** and **2b**. **3** was obtained from 3,4-(methylendioxy)phenol according to the method of Woods.[28]

Synthesis of 4′-substituted psoralens (Scheme 2) was achieved in two steps by modifying the method of MacLeod.[29] 7-Hydroxycoumarins were converted to the *β*-keto ethers **4a**−**9a** by reaction with chloroacetone or *ω*-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**.

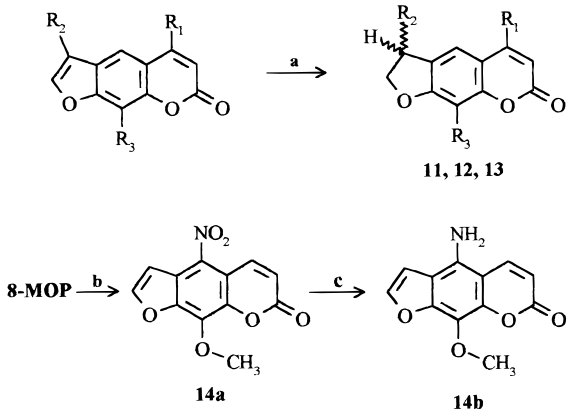
The 4′,5′-dihydropsoralen derivatives **11**−**13** were obtained by transfer hydrogenation of the necessary psoralens according to the method of Heindel[30] using cyclohexene in refluxing ethanol as a hydrogen donor

Scheme 3. Synthesis of Psoralen **10d**[a]



[a] Reagents: (a) Mg/I_2; (b) CrO_3, acetic acid; (c) Zn/HCl; (d) $C_2H_5OSO_2OC_2H_5$, acetone, K_2CO_3.

Scheme 4[a]



[a] Reagents: (a) cyclohexene, 10% Pd/C, ethanol; (b) HNO_3, acetic acid; (c) Zn/HCl.

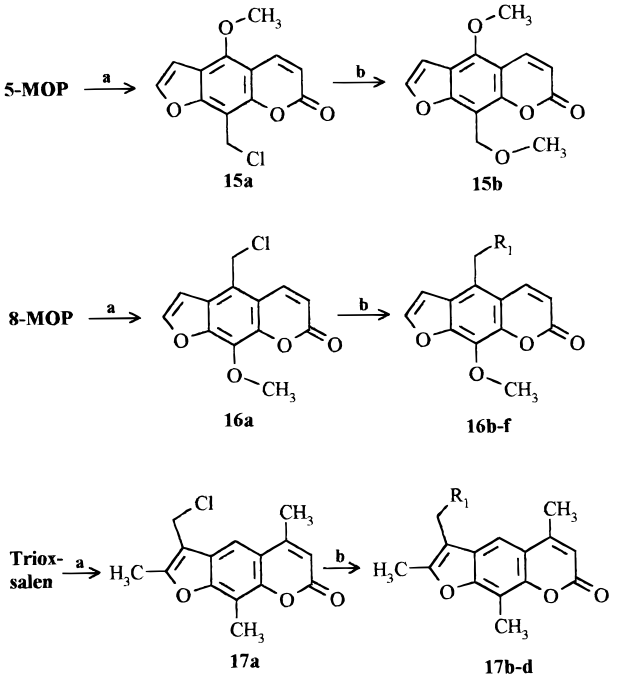
under catalysis by Pd/C (Scheme 4). Nitration of 8-MOP gave **14a**, and subsequent reduction gave **14b**.

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.[31] 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;[32] the slight differences probably reflect the fact that the channels from *Xenopus* are heteromultimers of Kv1.1 and another Kv1 α-subunit. The screening technology has been described previously.[19,33,34] Briefly, the blockade of steady-state

Scheme 5. Preparation of Alkoxymethylpsoralens[a]



[a] Reagents: (a) $ClCH_2OCH_3$, acetic acid; (b) alcohol or alkylamine.

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 μM, 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 alkoxy 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.

Table 1. Compound Structure and Block of Steady-State Axonal K^+ Currents[a]

compd	R_1	R_2	R_3	B_K, at 25 μM (%)	S
1b	H	C_2H_5		40	0.7
2b	CH_2OCH_3	C_2H_5		35	1.8
5-MOP				52	14
8-MOP				5	4
4b	CH_2OCH_3	CH_3		52	17
5b	CH_2OCH_3	Ph		27[b]	2
6b	H	CH_3		63	15
7b	CH_3	CH_3		25	8
8b	H	Ph		68[b]	1.4
9b	CH_2OH	CH_3		11	4
10d				75	14
11	H	H	OCH_3	4	5
12	H	CH_3	H	23	3
13	CH_3	CH_3	H	45	22
15b				35	26
16b	OCH_3			14	3
16c	OC_2H_5			16	2
16d	$OCH(CH_3)_2$			10	3
16e	$OCH_2CH{=}CH_2$			24	6
16f	$N^+(C_2H_5)_3$			20[c]	7
17b	OCH_3			31	11
17c	OC_2H_5			55	6
17d	$N^+(C_2H_5)_3$			27[c]	5

[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 μM. [c] Tested at 0.5 mM.

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.

5-MOP, **4b**, and **10d** showed the highest affinity for Kv1.2. However a variation of substitution pattern also changes the sensitivity of the different *Shaker* channels. Compound **4b**, bearing substituents in 4- and 4′-positions, shows the highest affinity to Kv1.1 and Kv1.2, while 5,8-disubstituted psoralens such as **10d** are equally effective blockers of Kv1.2 and Kv1.3. Consis-

Table 2. Sensitivity of K^+ Channels of the *Shaker* and *Shaw* Families to Block[a]

K_d (μM)	Kv1.1	Kv1.2	Kv1.3	Kv1.5	Kv1.6	Kv3.1
4-AP[35]	290	590	195	270	nd	29
5-MOP	>200	41	101	177	187	111
4b	56	18	90	nd	nd	213
10d	167	23	28	nd	nd	178
15b	nd	nd	28	nd	nd	nd

[a] K_d, 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

	induced ΔT_m shift (°C)[a]		
compd	dark	UV-A irradiated	$^1O_2$ production[b] (%)
5-MOP	0.5	13	5
8-MOP	1	10	9
5,8-diMOP	0.5	1	−5
4b	0.5	>20	43
6b	1	9	71
10d	0.5	0.5	−4
12	0.5	0.5	100
15b	1	1	4
16c	2	1.5	7
17b	0.5	>20	50
17c	1	>20	50
17d	7	16	66

[a] Mean value (±0.5 °C) from three determinations for a [compound]:[DNA] ratio of 1:1. [b] 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).

tent with this hypothesis, **15b** was found to have similar potency to **10d** in blocking Kv1.3. The apamin-sensitive SK_{Ca} channel from Jurkat T-cells was unaffected even by 100 μM 5-MOP.

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 photoreactivity 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 quarternary 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 $^1O_2$ by various compounds (10 μM) was determined in O_2-saturated solutions according to the method of Kraljic and El Mohsni,[39] which is based on the bleaching of *N*,*N*-dimethyl-*p*-nitrosoaniline (50 μM) by $^1O_2$ 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. 4′-Methylpsoralens (**4b** and **6b**), dihydropsoralens (**12**), and derivatives of trioxsalen (**17b**,**c**) were found to be effective producers of $^1O_2$. As expected the reference compounds 5- and 8-MOP[40] were only weak producers of $^1O_2$; their derivatives **15b** and **16c** also exhibited low activity. 5,8-Dialkoxypsoralens such as 5,8-dimethoxypsoralen (isopimpinellin) and **10d** were found to completely lack the ability to generate $^1O_2$; 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.

Table 4. In Vitro Phototoxicity on *A. salina*

	LC_{50} (μM)		photoinduced
compd	daylight	UV-A irradiated	dose modification
5-MOP	>100	2.5	>40
8-MOP	>100	2.5	>40
5,8-diMOP	>100	>100	
4b	>100	2.5	>40
6b	>100	0.5	>200
7b	>100	0.7	>150
10d	>100	>100	
12	>100	25	>4
13	>100	25	>4
15b	>100	>100	
16b	>100	>100	
17b	>100	20	>5

Photobiological Activity. 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 $^1O_2$,[42] 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] 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

of psoralens from their phototoxic and photomutagenic properties. Its nearly equivalent affinity to Kv1.2 and Kv1.3 makes **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 **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.[45] 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 melting point 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 Perkin-Elmer 1600 series FTIR (KBr disks, ν in 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 ($CHCl_3$ in $CDCl_3$ at 7.24 ppm and DMSO in DMSO-d_6 at 2.49 ppm) or TMS. Signals are designated as follows: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quadruplet), m (multiplet), ex (exchangeable with D_2O). ^{13}C 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 ^1H NMR and MS spectra of known compounds were in agreement with their chemical structure.

7-Hydroxy-4-(methoxymethyl)coumarin was purchased from Maybridge. Compounds **3**,[28] **9b**,[47] **10a**,[48] **10b**,[49] **14a**,[54] **14b**,[55] **16a**−**e**,[57] **16f**,[58] and **17a**,**b**[56] were synthesized as described previously. Compounds **6a**,[46] **7a**,[29] and **8a**[29] were prepared according to general method A; compounds **6b**,[46] **7b**,[29] and **8b**[29] 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). After stirring under reflux for 24 h the mixture was poured into HCl (5%, 100 mL), concentrated under reduced pressure to one-half its volume, and at kept at 4 °C overnight. The precipitate was collected by vacuum filtration and recrystallized from ethanol (9.6 g, 68%): mp 280 °C (lit.[27] mp 280 °C).

5,7-Diethoxycoumarin (1b). To a solution of **1a** (1.0 g, 5.6 mmol) in anhydrous acetone (100 mL) were added potassium carbonate (5 g) and iodoethane (2 mL). After being heated under reflux 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 ethanol (996 mg, 76%): mp 116 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.98 (d, 1H, ^3J = 9.7 Hz, 4-H), 6.54 (d, ^4J = 1.9 Hz, 1H, H-8), 6.46 (d, ^4J = 1.9 Hz, 1H, 6-H), 6.17 (d, 1H, ^3J = 9.7 Hz, 3-H), 4.13 (m, 4H, 2 × $-OCH_2CH_3$), 1.36 (m, 6H, 2 × $-OCH_2CH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.65 (C-7), 162.25 (C-2), 157.43 (C-5), 156.93 (C-8a), 139.55 (C-4), 111.24 (C-3), 104.59 (C-4a), 96.34 (C-6), 93.79 (C-8), 64.98 and 64.77 (5- and 7-OCH_2CH_3), 15.17 (5- and 7-OCH_2CH_3); MS *m*/*z* 234 (M^+, 66), 206 (28), 178 (60), 150 (100), 121 (12). Anal. ($C_{13}H_{14}O_4$) C, H.

5,7-Dihydroxy-4-(methoxymethyl)coumarin (2a). To phloroglucinol dihydrate (5.0 g, 30 mmol) in concentrated H_2SO_4 (100 mL) at 0 °C was added methyl 4-methoxyacetoacetate (4.4 g, 30 mmol) dropwise. The mixture was stirred for 10 h at room temperature, then poured into cold water (500 mL), and kept at 4 °C overnight. The precipitate formed was collected by vacuum filtration, washed with water, and recrystallized from ethanol (2.53 g, 38%): mp 220 °C; ^1H NMR (300 MHz, DMSO) δ 10.68 and 10.31 (2s, 2H, ex, 5- and 7-OH), 6.26 (d, 1H, ^4J = 2.2 Hz, 8-H), 6.20 (d, 1H, ^4J = 2.2 Hz, 6-H), 6.05 (s, 1H, 3-H), 4.74 (s, 2H, 4 $-CH_2OCH_3$), 2.51 (s, 3H, 4-CH_2OCH_3); ^{13}C NMR (75 MHz, DMSO) δ 161.20 (C-2), 160.38 (C-7), 157.25 (C-5), 156.37 (C-8a), 155.19 (C-4), 103.79 (C-3), 99.95 (C-4a), 98.94 (C-6), 94.74 (C-8), 71.59 (4-CH_2OCH_3), 58.43 (4-CH_2OCH_3); MS *m*/*z* 222 (M^+, 63), 190 (100), 162 (37), 151 (14), 134 (27), 77 (20), 69 (63). Anal. ($C_{11}H_{10}O_5$) C, H.

5,7-Diethoxy-4-(methoxymethyl)coumarin (2b). Compound **2b** was prepared from **2a** (0.50 g, 2.3 mmol) according to the method given for **1b** (617 mg, 74%): mp 143 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 6.43 (d, 1H, ^4J = 2.2 Hz, 8-H), 6.42 (d, 1H, ^4J = 2.2 Hz, 6-H), 6.25 (s, 1H, 3-H), 4.76 (s, 2H, 4-CH_2OCH_3), 4.06 (m, 4H, 5- and 7-OCH_2CH_3), 3.50 (s, 3H, 4-CH_2OCH_3)1.46 (m, 6H, 5- and 7-OCH_2CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.13 (C-2), 161.37 (C-7), 157.62 (C-5), 156.82 (C-8a), 154.42 (C-4), 106.62 (C-3), 102.57 (C-4a), 96.03 (C-6), 94.02 (C-8), 72.42 (4-CH_2OCH_3), 64.72 and 64.09 (5- and 7-OCH_2CH_3), 58.97 (4-CH_2OCH_3), 14.56 (5- and 7-OCH_2CH_3); MS *m*/*z* 278 (M^+, 100), 263 (7), 249 (8), 233 (56), 220 (10), 206 (36), 190 (17), 179 (16), 163 (12), 151 (9). Anal. ($C_{15}H_{18}O_5$) C, H.

General Method A: Preparation of 7-(2-Oxopropoxy)- and 7-(Phenacyloxy)coumarins. To a solution of the required 7-hydroxycoumarin (20.0 mmol) in anhydrous acetone (100 mL) were added potassium carbonate (10 g) and chloroacetone (1.8 mL, 20.0 mmol). After being heated 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[29]).

General Method B: Preparation of 4′-Methyl- and 4′-Phenylpsoralens. The required 7-(2-oxypropoxy)- or 7-(phenacyloxy)coumarin (10 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; for the 4′-phenylpsoralens a mixture of methanol and ethyl acetate (4:1) was used (modified after MacLeod[29]).

5,7-Bis(2-oxopropoxy)coumarin (1c). Compound **1c** was synthesized from **1a** (1.4 g, 7.8 mmol) and chloroacetone (0.90 mL, 16 mmol) according to general method A (1.80 g, 82%): mp 188 °C; ^1H NMR (300 MHz, DMSO) δ 4.98 and 4.75 (2 × s, 4H, 5- and 7-OCH_2COCH_3), 2.20 and 2.16 (2 × s, 6H, 5- and 7-OCH_2COCH_3); ^{13}C NMR (75 MHz, DMSO) δ 203.12 and 202.90 (5- and 7-OCH_2COCH_3), 72.75 and 72.39 (5- and 7-OCH_2COCH_3), 26.27 and 26.14 (5- and 7-OCH_2COCH_3); MS *m*/*z* 290 (M^+, 62), 247 (45), 219 (25), 191 (12), 161 (8), 43 (100). Anal. ($C_{15}H_{14}O_6$) C,H.

4-(Methoxymethyl)-7-(2-oxopropoxy)coumarin (4a). Compound **4a** was synthesized from 4.8 mmol of 7-hydroxy-4-(methoxymethyl)coumarin according to general method A (993 mg, 79%): mp 104−105 °C; ^1H NMR (300 MHz, DMSO) δ 7.48 (d, 1H, ^3J = 8.7 Hz, 5-H), 6.86 (dd, 1H, ^3J = 8.7 Hz, ^4J = 2.3 Hz, 6-H), 6.77 (d, 1H, ^4J = 2.3 Hz, 8-H), 6.39 (s, 1H, 3-H), 4.64 (s, 2H, 4-CH_2OCH_3), 4.57 (s, 2H, 7-OCH_2COCH_3), 3.49 (s, 3H, 4-CH_2OCH_3), 2.30 (s, 3H, 7-OCH_2COCH_3); ^{13}C NMR (75 MHz, DMSO) δ 203.04 (7-OCH_2COCH_3), 160.81 (C-7), 160.50 (C-2), 154.80 (C-8a), 125.71 (C-5), 112.42 (C-3), 110.93 (C-4a), 108.99 (C-6), 101.49 (C-8), 72.25 (7-OCH_2COCH_3), 69.34 (4-CH_2OCH_3), 58.31 (4-CH_2OCH_3), 26.11 (7-OCH_2COCH_3); MS *m*/*z* 262 (M^+, 45), 232 (22), 219 (31), 205 (12), 191 (18), 131 (13), 43 (100). Anal. ($C_{14}H_{14}O_5$) C, H.

4-(Methoxymethyl)-4′-methylpsoralen (4b). Compound **4b** was prepared from **4a** (0.8 mmol) according to general method B (127 mg, 65%): mp 148 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.65 (s, 1H, 5-H), 7.47 (s, 1H, 5′-H), 7.43 (s, 1H, 8-H), 6.51 (s, 1H, 3-H), 4.72 (s, 2H, 4-CH_2OCH_3), 3.54 (s, 3H, 4-CH_2-

OCH_3), 2.28 (s, 3H, 4′-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.11 (C-2), 156.57 (C-7), 151.83 (C-8a), 151.68 (C-4), 143.25 (C-5′), 126.48 (C-4′), 115.66 (C-6), 113.94 (C-5), 113.61 (C-4a), 111.44 (C-3), 99.95 (C-8), 70.64 (4-CH_2OCH$_3$), 59.07 (4-CH_2OCH_3), 7.84 (4′-CH_3); MS *m*/*z* 244 (M^+, 100), 229 (3), 216 (22), 201 (10), 185 (71), 173 (46), 157 (7), 145 (57), 128 (23). Anal. ($C_{14}H_{12}O_4$) C, H.

4-(Methoxymethyl)-7-(phenacyloxy)coumarin (5a). Compound **5a** was synthesized from 7-hydroxy-4-(methoxymethyl)-coumarin (1.0 g, 4.8 mmol) and ω-chloroacetophenone (0.75 g, 4.8 mmol) according to general method A (1290 mg, 83%): mp 184 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05−7.02 (m, 8H, 7-OCH$_2$COC$_6H_5$ and 5-H, 8-H, and 6-H), 6.27 (s, 1H, 3-H), 4.67 (s, 2H, 4-CH_2OCH$_3$), 3.41 (s, 2H, 7-OCH_2COC$_6$H$_5$), 3.31 (s, 3H, 4-CH_2OCH_3); MS *m*/*z* 324 (M^+, 25), 105 (100), 91 (8), 77 (25). Anal. ($C_{19}H_{16}O_5$) C, H.

4-(Methoxymethyl)-4′-phenylpsoralen (5b). Compound **5b** was prepared from 4.0 mmol of **5a** according to general method B (771 mg, 63%): mp 191 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (s, 1H, 5-H), 7.81 (s, 1H, 5′-H), 7.63−7.41 (m, 6H, 4′-C$_6H_5$ and 8-H), 6.53 (s, 1H, 3-H), 4.69 (s, 2H, 4-CH_2-OCH$_3$), 3.53 (s, 3H, 4-CH_2OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 130.90 (C-1″), 129.20 (C-3″ and C-5″), 128.02 (C-4″), 127.49 (C-4″ and C-6″), 123.91 (C-4′), 122.22 (C-6); MS *m*/*z* 306 (M^+, 100), 278 (21), 247 (49), 235 (19), 214 (10), 207 (41), 189 (25), 178 (27). Anal. ($C_{19}H_{14}O_4$) C, H.

5,8-Dihydroxypsoralen (10c). 10b (500 mg, 2.3 mmol) and 3 g of zinc dust were suspended in a mixture of acetone (30 mL) and water (100 mL) at room temperature; 10 mL of concentrated HCl was added dropwise. After 5 h the remaining zinc dust was filtered off, and the filtrate was concentrated under reduced pressure to one-half its volume and kept at 4 °C overnight. The precipitate was collected by vacuum filtration and recrystallized from water (311 mg, 62%): mp 268 °C (lit.[51] mp 270 °C).

5,8-Diethoxypsoralen (10d). To a solution of 200 mg (0.9 mmol) of **10c** in anhydrous acetone (100 mL) were added potassium carbonate (5 g) and diethyl sulfate (1.0 mL, 3.6 mmol). The mixture was heated to reflux under nitrogen; after 3 h it was poured into cold water (200 mL) and acidified with HCl. After 24 h the product was extracted with ethyl acetate (3 × 100 mL), the organic layers were combined, and the solvent was removed under reduced pressure to afford a residue, which was purified by column chromatography using 3:1 ethyl acetate/cyclohexane as eluent. The desired fractions were concentrated and dried to give **10d** as a yellowish powder (158 mg, 64%): mp 119 °C (lit.[52] mp 105 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13 (d, 1H, ^3J = 9.8 Hz, 4-H), 7.61 (d, 1H, ^3J = 2.2 Hz, 5′-H), 6.92 (d, 1H, ^3J = 2.2 Hz, 4′-H), 6.28 (d, 1H, ^3J = 9.8 Hz, 3-H), 4.40 (two q, 4H, 5- and 8-OCH_2CH$_3$), 1.47 (two t, 6H, 5- and 8-OCH$_2$CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.65 (C-2), 150.41 (C-7), 145.11 (C-5′), 144.08 (C-8a), 143.51 (C-5), 139.65 (C-4), 127 (C-8), 115.55 (C-6), 112.78 (C-3), 108.27 (C-4a), 105.16 (C-4′), 69.95 and 69.43 (5- and 8-OCH_2CH$_3$), 15.62 (5- and 8-OCH$_2$CH_3); MS *m*/*z* 274 (M^+, 55), 246 (24), 217 (100), 189 (17), 161 (13), 133 (6), 105 (8), 77 (10). Anal. ($C_{15}H_{14}O_5$) C, H.

General Method C: Transfer Hydrogenation of Psoralens. To a solution of the required psoralen (1 g) in ethanol (200 mL) were added cyclohexene (5 mL) and a suspension of 10% palladium on carbon (1 g) in ethanol (20 mL). After the mixture was stirred and heated to reflux for 36 h, the catalyst was filtered off and the solvent was removed under reduced pressure to afford a residue, which was recrystallized from methanol (modified after Heindel[30]).

Compounds **11**[30,51] (from 8-MOP) and **12**[53] (from **6b**) were prepared according to general method C.

4′,5′-Dihydro-4,4′-dimethylpsoralen (13). Compound **13** was prepared from **7b** (1.0 g, 4.7 mmol) according to general method C (727 mg, 72%): mp 146 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (s, 1H, 5-H), 7.01 (s, 1H, 8-H), 6.11 (s, 1H, 3-H), 4.80 (t, 1H, 5′-H) and 4.20 (q, 1H, 5′-H), 3.59 (m, 1H, ^3J = 6.8 Hz, 4′-H), 2.40 (s, 3H, 4-CH_3), 1.37 (d, 3H, ^3J = 6.8 Hz, 4′-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.80 (C-7), 162.04 (C-2), 155.73 (C-8a), 153.32 (C-4), 130.61 (C-6), 119.84 (C-5), 114.32 (C-4a), 111.75 (C-3), 98.71 (C-8), 80.59 (C-5′), 36.27 (C-4′), 20.18 (4′-CH_3), 19.57 (4-CH_3); MS *m*/*z* 216 (M^+, 93), 201 (100), 188 (20), 173 (80), 145 (60), 117 (14), 115 (34). Anal. ($C_{13}H_{12}O_3$) C, H.

Chloromethylation of Psoralens. Caution: Chlorodimethyl ether is lachrymatory and highly carcinogenic; therefore the following experiments must be conducted with extreme caution. As chloromethylated compounds are highly sensitive to hydrolysis, **15a**, **16a**, and **17a** were only characterized by ^{1}H NMR and MS.

8-(Chloromethyl)-5-methoxypsoralen (15a). Compound **15a** was prepared from 5-MOP (0.50 g, 2.3 mmol) according to the method of Isaacs[56] given for the chloromethylation of trioxsalen (407 mg, 67%): mp 200 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 (d, 1H, ^3J = 9.8 Hz, 4-H), 7.64 (d, 1H, ^3J = 2.2 Hz, 5′-H), 7.04 (d, 1H, ^3J = 2.2 Hz, 4′-H), 6.30 (d, 1H, ^3J = 9.8 Hz, 3-H), 5.07 (s, 2H, 8-CH_2Cl), 4.29 (s, 3H, 5-OCH_3); MS *m*/*z* 264 (M^+, 26), 229 (100), 201 (19), 186 (24).

5-Methoxy-8-(methoxymethyl)psoralen (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; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.91 (s, 2H, 8-CH_2OCH$_3$), 4.26 (s, 3H, 5-OCH_3), 3.38 (s, 3H, 8-CH_2OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.80 (C-2), 157.74 (C-7), 150.80 (C-8a), 149.49 (C-5), 144.90 (C-5′), 139.33 (C-4), 112.27 (C-3), 106.18 (C-6), 105.51 (C-5), 105.15 (C-4′), 103.47 (C-8), 62.18 (8-CH_2OCH$_3$), 60.03 (5-OCH_3), 58.44 (8-CH_2OCH_3); MS *m*/*z* 260 (M^+, 39), 229 (100), 201 (23), 186 (24), 173 (6), 158 (5). Anal. ($C_{14}H_{12}O_5$) 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; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.61 (s, 1H, 5-H), 6.24 (s, 1H, 3-H), 4.62 (s, 2H, 4′-CH_2OCH$_2$CH$_3$), 3.55 (q, 2H, 4′-CH_2OCH_2CH$_3$), 2.57−2.49 (3 × s, 9H, 4-CH_3, 5′-CH_3 and 8-CH_3), 1.25 (t, 3H, 4′-CH_2OCH$_2$CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) 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), 109.01 (C-8), 65.47 (4′-CH_2OCH$_2$-CH$_3$), 62.79 (4′-CH_2OCH_2CH$_3$), 19.35 (5′-CH_3), 15.24 (4′-CH_2-OCH$_2$CH_3), 12.33 (4-CH_3), 8.47 (8-CH_3); 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. ($C_{17}H_{18}O_4$) 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 Hansen[58] given for **16f** (247 mg, 91%): mp 214 °C; ^{1}H NMR (300 MHz, DMSO) δ 7.02 (s, 1H, 5-H), 6.40 (s, 1H, 3-H), 4.80 (s, 2H, 4′-CH_2N$^+$(CH$_2$CH$_3$)$_3$), 3.33 (m, 6H, 4′-CH_2N$^+$(CH_2CH$_3$)$_3$), 2.75−2.50 (3 × s, 9H, 4-CH_3, 5′-CH_3, 8-CH_3), 1.34 (t, 9H, 4′-CH_2N$^+$(CH$_2$CH_3)$_3$); ^{13}C NMR (75 MHz, DMSO) δ 159.83 (C-5′), 124.89 (C-4′), 104.57 (4′-CH_2N$^+$(CH$_2$CH$_3$)$_3$), 52.83 (4′-CH_2N$^+$(CH_2CH$_3$)$_3$), 18.81 (5′-CH_3), 13.28 (4-CH_3), 8.10 (4′-CH_2N$^+$(CH$_2$CH$_3$)$_3$), 7.91 (8-CH_3); MS (thermospray, LC/MS) *m*/*z* 378 (MH^+, 21), 342 (29), 201 (15), 277 (40), 258 (39), 241 (86), 102 (100). Anal. ($C_{21}H_{28}O_3$NCl) C: calcd, 66.74; found, 66.31. H: calcd, 7.46; found, 7.50. N: calcd, 3.71; found, 3.71.

UV-A Irradiation. Irradiations were performed by means of a 150-W Heraeus Fluotest mercury-vapor lamp; irradiation intensities, determined with a Holtkamp UV-A meter 360, were 4.5 and 1.3 mW/cm^2 at a distance of 20 and 45 cm, respectively.

Thermal Transition Studies. Melting studies were performed in 2-mL quartz cuvettes using a HP 8845A diode-array spectrophotometer fitted with a Julabo 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 (T_m) were determined at the midpoint of the

melting profiles. Drug-induced alterations in the DNA melting behavior are given by: $\Delta T_m = T_m(\text{DNA + drug}) - T_m(\text{DNA})$, where the T_m for the drug-free DNA control is 34.0 ± 0.2 °C; results are given as the mean from three determinations.

A stock solution containing 1 unit of the polynucleotide in 100 μL 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 μM DNA (20 μL of the stock solution). DNA−drug solutions were prepared by addition of the compounds from a stock solution in methanol (10^{-3} M) to give a final drug concentration of 10 μM. 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 μL 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 phototropic nauplii were collected using a Pasteur pipet. Ten shrimps were transferred to each sample vial containing 3 mL of seawater and 100 nM, 500 nM, 1 μM, 2.5 μM, 5 μM, 10 μM, 25 μM, 50 μM, or 100 μM 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 nontoxic 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.

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