Paul Körner

Pharmazeutisches Institut,
Universität Kiel, Kiel, Germany

3-Alkyl- and 3-Aryl-*7H*-furo[3,2-*g*]-1-benzopyran-7-ones:

Synthesis, Photoreactivity, and Fluorescence Properties

A series of 3-alkyl- and 3-aryl-*7H*-furo[3,2-*g*]-1-benzopyran-7-ones, known as linear furocoumarins, was synthesized and evaluated for their dark- and photobinding (crosslink formation) with DNA as well as for their spectrophotometric and fluorescent properties, lipophilicity, and ability to photobleach *N,N*-dimethyl-*p*-nitrosoaniline (RNO) after irradiation with UVA light. 8-Methoxypsoralen (8-MOP, 9-methoxy-*7H*-furo[3,2-*g*]-1-benzopyran-7-one) and 4,5′,8-trimethylpsoralen (TMP, 2,5,9-trimethyl-*7H*-furo[3,2-*g*]-1-benzopyran-7-one) were used as reference compounds in all tests. The investigations support the formation of a molecular complex between the furocoumarins and DNA. Crosslink formation with DNA after irradiation with UVA light was detectable for compounds with a methyl or phenyl substituent in position 3, but not for those bearing either a *tert*-butyl, a 4-methoxyphenyl, or a 2,5-dimethoxyphenyl group. All furocoumarins exhibited sufficient absorption in the UVA wavelength range and are fluorescent. All compounds showed a higher lipophilicity than 8-MOP. Generally the 3-alkyl substituted furocoumarins had a capacity to photobleach RNO which was higher than that of the 3-aryl substituted ones. Some of the 3-aryl substituted furocoumarins displayed a photobleaching ability which was similar to or lower than that of 8-MOP.

Keywords: Furocoumarins; DNA intercalation; Fluorescence; Lipophilicity; Photoreactivity

Received: August 8, 2001 [FP619]

Full Paper

Introduction

Furocoumarins (psoralens, *7H*-furo[3,2-*g*]-1-benzopyran-7-ones) are widely used in PUVA therapy (psoralen plus UVA irradiation) as photoreactive drugs in the treatment of various skin diseases such as psoriasis, mycosis fungoides, vitiligo [1–3], and in photopheresis, an extracorporal form of photochemotherapy [4, 5]. The derivatives, which are usually employed for both PUVA and photopheresis, are 8-methoxypsoralen (8-MOP), 5-methoxypsoralen (5-MOP), and 4,5′,8-trimethylpsoralen (TMP) [6]. The biological activity of these furocoumarins has been correlated to their ability to photoreact with DNA [2]. The planar compounds are able to intercalate between two base pairs of a DNA double helix in the dark. Upon UVA irradiation, mono- and diadducts – interstrand crosslinks – can be formed. This DNA photodamage is responsible for some of the desired antiproliferative effects but also for the undesired side-effects such as carcinogenicity, genotoxicity, and the formation of skin erythemas [2]. Both mono- and diadducts are effective in inhibiting DNA synthesis, although decreased photoinduced mutagenicity has been found in the case of monofunctional as opposed to bifunctional furocoumarins [7]. For this reason monofunctional furocoumarins have been prepared and investigated by several groups [2, 8–12] with the aim of reducing these undesired side effects. It has also been shown that the reactivity of the double bonds can be modified by substitution with methyl groups, thus influencing charge donation, steric effects and the lipophilic nature of the furocoumarins [13]. A further field of interest is the responsible mechanism of PUVA. Furocoumarins also react with proteins and membrane lipids, either by direct photoaddition or by generation of singlet oxygen, which suggests that parallel to the DNA reaction other cellular targets should be taken into account when attempting to explain furocoumarin photosensitization [14]. Because of the diversity of furocoumarin reactivity, the development of new derivatives with specific target oriented properties is still an important task [2].

Correspondence: Paul Körner, Pharmazeutisches Institut, Universität Kiel, Gutenbergstr. 76, D-24118 Kiel, Germany. Phone: +49 (0)431 880 1137, Fax: +49 (0)431 8801352, e-mail: pKoerner@pharmazie.uni-kiel.de.

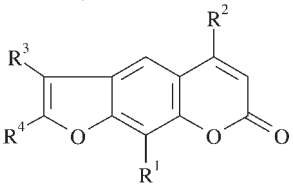
 0365-6233/05/0187 $ 17.50+.50/0

The author was interested in the preparation and investigation of furocoumarins which have hydrophobic and bulky substituents on the furan moiety, in order to check the influence of the structural changes in position 3 of the furocoumarin skeleton on the interaction with DNA as well as on the absorption and fluorescence spectra, the lipophilicity, and the photobleaching effect of *N,N*-dimethyl-*p*-nitrosoaniline. This method has been used by several groups for measuring the generation of singlet oxygen [6, 10, 15–19].

The present paper describes the preparation of five series of furocoumarins. They consisted of two series of 3-alkyl derivatives (3-methyl (**3 a**–**c**) and 3-*tert*-butyl (**3 d**–**f**)) and three series of 3-aryl derivatives (3-phenyl (**3 g**–**i**), 3-(4-methoxyphenyl) (**3 j**–**l**), and 3-(2,5-dimethoxyphenyl) (**3 m**–**o**)).

Linear furocoumarins with a 3-methyl (**3 a**–**c**) or 3-phenyl (**3 g**–**h**) group at the furan moiety have also been prepared by several groups in the past [20–22]. In this present work, in a comparative study, these compounds are investigated together with newly synthesised derivatives containing a 3-*tert*-butyl-, a 3-(4-methoxyphenyl)- or a 3-(2,5-dimethoxyphenyl) group. 8-MOP and TMP were used as reference compounds in all tests.

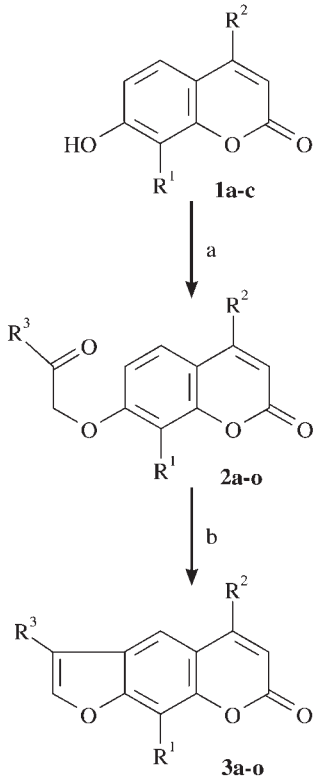
Table 1. Substitution pattern of furocoumarins (**3**).



Compound	R^1	R^2	R^3	R^4
3 a	H	H	Me	H
3 b	H	Me	Me	H
3 c	Me	Me	Me	H
3 d	H	H	*tert*-Bu	H
3 e	H	Me	*tert*-Bu	H
3 f	Me	Me	*tert*-Bu	H
3 g	H	H	Ph	H
3 h	H	Me	Ph	H
3 i	Me	Me	Ph	H
3 j	H	H	4-MeOPh	H
3 k	H	Me	4-MeOPh	H
3 l	Me	Me	4-MeOPh	H
3 m	H	H	2,5-$(MeO)_2$Ph	H
3 n	H	Me	2,5-$(MeO)_2$Ph	H
3 o	Me	Me	2,5-$(MeO)_2$Ph	H
8-MOP	MeO	H	H	H
TMP	Me	Me	H	Me

Chemistry

The furocoumarins (**3 a**–**o**) were obtained from the corresponding 7-hydroxy-*2H*-1-benzopyran-2-ones (**1 a**–**c**), as indicated in Scheme 1. 7-Hydroxy-*2H*-1-benzopyran-2-one (**1 a**) and 4-methyl-7-hydroxy-*2H*-1-benzopyran-2-one (**1 b**) are commercially available compounds. 4,8-Dimethyl-7-hydroxy-*2H*-1-benzopyran-2-one (**1 c**) [23] was prepared via the Pechmann reaction. The synthesis of furocoumarins was then achieved in two steps by modifying the method of MacLeod [21]. The 7-hydroxy-*2H*-1-benzopyran-2-ones (**1 a**–**c**) were converted into β-ketoethers (**2 a**–**o**) by alkylation with the appropriate haloketone under phase-transfer catalysis conditions. Base-catalyzed intramolecular condensation in 1 N aqueous KOH under nitrogen for 8 h and subsequent acidification gave the corresponding linear furocoumarins (**3 a**–**o**). Following this synthetic route, several compounds, bearing different substituents R^3 (R^3 = methyl, *tert*-butyl, phenyl, 4-methoxyphenyl, 2,5-dimeth-



R^1 = H, CH_3

R^2 = H, CH_3

R^3 = CH_3 , $C(CH_3)_3$, C_6H_5, 4-CH_3O-C_6H_4, 2,5-$(CH_3O)_2C_6H_3$

Scheme 1. a) acetone, K_2CO_3 , 18-crown-6, reflux, R^3-CO-CH_2-X, X = Cl, Br; b) 1. 1 N KOH, reflux, 2.30 % H_3PO_4 .

Table 2. Some characteristics of compounds **2d**–**f**, **2i**, **2k**–**o**.

Comp. No.	Yield (%)	Mp[a] (°C)	Formula	MW	Anal. Calcd.: Found:
2d	45	98	$C_{15}H_{16}O_4$	260.29	C, 69.22; H, 6.20 C, 68.90; H, 6.13
2e	40	116	$C_{16}H_{18}O_4$	274.31	C, 70.06; H, 6.61 C, 69.85; H, 6.66
2f	40	123	$C_{17}H_{20}O_4$	288.34	C, 70.81; H, 6.99 C, 70.86; H, 7.17
2i	62.5	198	$C_{19}H_{16}O_4$	308.33	C, 74.01; H, 5.23 C, 73.63; H, 5.13
2k	82	158	$C_{19}H_{16}O_5$	324.33	C, 70.36; H, 4.97 C, 70.29; H, 5.06
2l	77	200	$C_{20}H_{18}O_5$	338.36	C, 71.00; H, 5.36 C, 70.50; H, 5.34
2m	83	176	$C_{19}H_{16}O_6$	340.33	C, 67.06; H, 4.74 C, 66.81; H, 4.90
2n	92	182	$C_{20}H_{18}O_6$	354.36	C, 67.79; H, 5.12 C, 67.73; H, 5.32
2o	75	205	$C_{21}H_{20}O_6$	368.38	C, 68.47; H, 5.47 C, 68.38; H, 5.46

[a] Recrystallization solvent: ethanol.

Table 3. ^{1}H-NMR spectroscopic data and MS data of compounds **2d**–**f**, **2i**, **2k**–**o**.

Comp. No.	^{1}H-NMR; δ (ppm)	MS (70 eV): *m/z* (%)
2d	7.98 (d, ^{1}H, ^3J = 9.1 Hz, 4-H), 7.38 (d, 1H, ^3J = 9.3 Hz, 5-H), 6.93 (m, 2H, 8-H, 6-H), 6.3 (d, 1H, ^3J = 9.5 Hz, 3-H), 5.52 (s, 2H, $-OCH_2CO$), 1.19 (s, 9H, $-COC(CH_3)_3$)[a]	260 (21, M^+), 176 (24), 147 (20), 89 (12), 63 (8), 57 (100), 41 (25)
2e	7.65 (d, 1H, ^3J = 9.1 Hz, 5-H), 6.92 (m, 2H, 6-H, 8-H), 6.64 (q, 1H, ^4J = 1.2 Hz, 3-H), 5.51 (s, 2H, $-OCH_2CO$), 2.39 (d, 3H, ^4J = 1.2 Hz, 4-CH_3), 1.22 (s, 9H, $-COC(CH_3)_3$[a]	274 (37, M^+), 189 (47), 161 (28), 103 (9), 85 (8), 57 (100), 41 (24)
2f	7.38 (d, 1H, ^3J = 8.8 Hz, 5-H), 6.58 (d, 1H, ^3J = 8.8 Hz, 6-H), 5.0 (s, 2H, $-OCH_2CO$), 2.39 (s, 3H, 4-CH_3), 2.38 (s, 3H, 8-CH_3), 1.28 (s, 9H, $-COC(CH_3)_3$)[b]	288 (40, M^+), 204 (51), 189 (22), 175 (8), 145 (9), 115 (8), 91 (5), 77 (4), 57 (100), 51 (3), 41 (21)
2i	8.0 (d, 2H, ^3J = 7.6 Hz, C_6-2′,6′-H), 7.64 (dd, 1H, ^3J = 7.5 Hz, ^3J = 7.5 Hz, C_6-4′-H), 7.52 (dd, 2H, ^3J = 7.6 Hz, ^3J = 7.6 Hz, C_6-3′,5′-H), 7.37 (d, 1H, ^3J = 8.8 Hz, 5-H), 6.71 (d, 1H, ^3J = 8.8 Hz, 6-H), 6.15 (s, 1H, 3-H), 5.38 (s, 2H, $-OCH_2CO$), 2.38 (s, 6H, 4-CH_3, 8-CH_3)[b]	308 (19, M^+), 203 (5), 189 (10), 115 (4), 105 (100), 91 (10), 77 (29), 65 (6), 51 (7), 43 (1)

Table 3 (Continued)

Comp. No.	^{1}H-NMR; δ (ppm)	MS (70 eV): *m/z* (%)
2 k	7.98 (d, 2 H, ^3J = 8.8 Hz, 4-CH_3OC_6-*2′,6′-H*), 7.51 (d, 1 H, ^3J = 8.6 Hz, 5-H), 6.99 (d, 2 H, ^3J = 8.8 Hz, 4-CH_3OC_6-*3′,5′-H*), 6.94 (dd, 1 H, ^3J = 8.9 Hz, ^3J = 2.4 Hz, 6-H), 6.79 (d, 1 H, ^3J = 2.2 Hz, 8-H), 6.13 (s, 1 H, 3-H), 5.32 (s, 2 H, –OC*H*$_2$CO), 3.90 (s, 3 H, CH_3O), 2.38 (s, 3 H, 4-CH_3)	324 (7, M^+), 135 (100), 121 (5), 107 (7), 92 (6), 77 (17), 64 (4), 51 (4), 44 (6)
2 l	8.00 (d, 2 H, ^3J = 8.9 Hz, 4-CH_3OC_6-*2′,6′-H*), 7.55 (d, 1 H, ^3J = 8.9 Hz, 5-H), 7.10 (d, 2 H, ^3J = 8.9 Hz, 4-CH_3OC_6-*3′,5′-H*), 6.98 (d, 1 H, ^3J = 8.9 Hz , 6-H), 6.21 (d, 1 H, ^4J = 1.1 Hz, 3-H), 5.70 (s, 2 H, –OCH_2CO), 3.87 (s, 3 H, CH_3O), 2.38 (d, 3 H, 4-C*H*$_3$, ^4J = 1.1 Hz), 2.28 (s, 3 H, 8-CH_3)[b]	338 (5, M^+), 190 (2), 162 (3), 150 (5), 135 (100), 121 (9), 107 (9), 92 (9), 77 (25), 64 (6), 51 (6), 44 (61)
2 m	7.64 (d, 1 H, ^3J = 9.5 Hz, 4-H), 7.98 (d, 1 H, ^4J = 3.1 Hz, 2,5-$(CH_3O)_2C_6$-*6′-H*), 7.39 (d, 1 H, ^3J = 8.6 Hz, 5-H), 7.15 (dd, 1 H, ^4J = 3.2 Hz, ^3J = 9.0 Hz, 2,5-$(CH_3O)_2C_6$-*4′-H*), 6.99 (d, 1 H, ^3J = 9.0 Hz, 2,5-$(CH_3O)_2C_6$-*3′-H*), 6.93 (dd, 1 H, ^3J = 8.6 Hz , ^4J = 2.2 Hz, 6-H), 6.76 (d, 1 H, ^4J = 2.0 Hz, 8-H), 6.25 (d, 1 H, ^3J = 9.5 Hz, 3-H), 5.33 (s, 2 H, –OCH_2CO), 3.97 (s, 3 H, CH_3O), 3.81 (s, 3 H, CH_3O)[b]	340 (6, M^+), 165 (100), 150 (4), 135 (2), 122 (6), 107 (12), 89 (6), 77 (14), 63 (6), 51 (6), 44 (11)
2 n	7.54 (d, 1 H, ^3J = 8.8 Hz, 5-H), 7.49 (d, 1 H, ^4J = 3.2 Hz, 2,5-$(CH_3O)_2C_6$-*6′-H*) , 7.15 (dd, 1 H, ^4J = 3.2 Hz, ^3J = 9.1 Hz, 2,5-$(CH_3O)_2C_6$-*4′-H*), 7.01 (d, 1 H, ^3J = 9.1 Hz, 2,5-$(CH_3O)_2C_6$-*3′-H*), 6.96 (dd, 1 H, ^3J = 8.8 Hz , ^4J = 2.5 Hz, 6-H), 6.76 (d, 1 H, ^4J = 2.5 Hz, 8-H), 6.15 (d, 1 H, ^4J = 1.0 Hz, 3-H), 5.33 (s, 2 H, –OCH_2CO), 3.97 (s, 3 H, CH_3O), 3.81 (s, 3 H, CH_3O), 2.39 (d, 3 H, 4-CH_3)[b]	354 (6, M^+),165 (100), 150 (4), 135 (2), 122 (6), 107 (11), 91 (5), 77 (15), 65 (6), 51 (7), 44 (39)
2 o	7.45 (d, 1 H, ^4J = 2.4 Hz, 2,5-$(CH_3O)_2C_6$-*6′-H*), 7.35 (d, 1 H, ^3J = 8.7 Hz, 5-H), 7.13 (dd, 1 H, ^4J = 2.6 Hz, ^3J = 9.0 Hz, 2,5-$(CH_3O)_2C_6$-*4′-H*), 6.98 (d, 1 H, ^3J = 9.00 Hz, 2,5-$(CH_3O)_2C_6$-*3′-H*), 6.64 (d, 1 H, ^3J = 8.8 Hz, 6-H), 6.13 (s, 1 H, 3-H), 5.37 (s, 2 H, –OCH_2CO), 3.94 (s, 3 H, CH_3O), 3.80 (s, 3 H, CH_3O), 2.39 (s, 3 H, 4-CH_3), 2.36 (s, 3 H, 8-CH_3)[b]	368 (6, M^+), 190 (3), 165 (100), 150 (5), 122 (8), 107 (14), 91 (10), 77 (22), 65 (9), 51 (9), 44 (50)

[a] DMSO-d_6; [b] $CDCl_3$.

Table 4. Some characteristics of compounds **3 d**–**f**, **3 i**–**o**.

Comp. No.	Yield (%)	Mp[a] (°C)	Formula	MW	Anal. or HRMS Calcd.: Found:
3 d	56	144	$C_{15}H_{14}O_3$	242.27	242.09430 242.09415
3 e	51	152	$C_{16}H_{16}O_3$	256.30	256.10995 256.10983
3 f	60	193	$C_{17}H_{18}O_3$	270.33	270.12558 270.12550

Table 4. (Continued)

Comp. No.	Yield (%)	Mp[a] (°C)	Formula	MW	Anal. or HRMS Calcd.: Found:
3 i	66	191	$C_{19}H_{14}O_3$	290.31	C, 78.61; H, 4.86 C, 78.23; H, 4.86
3 j	53	199	$C_{18}H_{12}O_4$	292.29	292.07355 292.07360
3 k	75	192	$C_{19}H_{14}O_4$	306.32	C, 74.50; H, 4.61 C, 74.29; H, 4.61
3 l	72	231	$C_{20}H_{16}O_4$	320.34	C, 74.99; H, 5.03 C, 74.73; H, 5.00
3 m	77	197	$C_{19}H_{14}O_5$	322.31	C, 70.80; H, 4.38 C, 70.45; H, 4.37
3 n	75	204	$C_{20}H_{16}O_5$	336.34	C, 71.42; H, 4.79 C, 71.12; H, 4.81
3 o	64	179	$C_{21}H_{18}O_5$	350.37	350.11542 350.11520

[a] Recrystallization solvent: ethanol

Table 5. ^{1}H-NMR spectroscopic data, and MS data of compounds **3 d**–**f**, **3 i**–**o**.

Comp. No.	^{1}H-NMR; δ (ppm)	MS (70 eV): *m/z* (%)
3 d	7.82 (d, 1 H, 5-H, ^3J = 9.6 Hz), 7.77 (s, 1 H, 4-H), 7.42 (s, 1 H, 9-H), 7.40 (s, 1 H, 2-H), 6.36 (d, 1 H, 6-H, 3*J* = 9.6 Hz), 1.45 (s, 9 H, 3-C(CH_3)$_3$)[a]	242 (45, M^+), 227 (100), 199 (17), 128 (7), 115 (6), 99 (4), 85 (19), 77 (3), 63 (5), 57 (5), 41(5)
3 e	7.86 (s, 1 H, 4-H), 7.39 (s, 1 H, 2-H), 7.37 (s, 1 H, 9-H), 6.25 (s, 1 H, 6-H), 2.52 (d, 3 H, 5-CH_3), 1.46 (s, 9 H, 3-C(CH_3)$_3$)[a]	256 (45, M^+), 241 (100), 213 (17), 185 (7), 141 (6), 115 (4), 92 (19), 67 (3), 51 (5), 41 (5)
3 f	7.73 (s, 1 H, 4-H), 7.40 (s, 1 H, 2-H), 6.23 (s, 1 H, 6-H), 2.55 (s, 3 H, 9-CH_3), 2.52 (s, 3 H, 5-CH_3), 1.46 (s, 9 H, 3-C(CH_3)$_3$)[a]	270 (M^+), 255 (100), 227 (14), 128 (7), 115 (6), 99 (26), 77 (6), 67 (11), 63 (5), 43 (7)
3 i	7.85 (s, 1 H, 4 H), 7.83 (s, 1 H, 2 H), 7.65–7.40 (m, 5 H, –C_6H_5), 6.29 (s, 1 H, 6-H), 2.65 (s, 3 H, 9-CH_3), 2.51 (s, 3 H, 5-CH_3)[a]	290 (100, M^+), 262 (48), 233 (6), 219 (5), 202 (7), 189 (12), 178 (4), 165 (6), 131 (8), 101 (7), 89 (8), 76 (6)
3 j	7.85 (s, 1 H, 2-H), 7.81 (d, 1 H, 3*J* = 9.6 Hz, 5-H), 7.78 (s, 1 H, 4-H), 7.55 (d, 2 H, ^3J = 7.0 Hz, 4-CH_3OC_6-*2′,6′-H*), 7.50 (s, 1 H, 9-H), 7.05 (d, 2 H, ^3J = 7.0 Hz, 4-CH_3OC_6-*3′,5′-H*), 6.4 (d, 1 H, ^3J = 9.6 Hz, 6-H), 3.88 (s, 3 H, OCH_3)[a]	292 (100, M^+), 277 (29), 249 (26), 165 (15), 132 (9), 88 (9), 44 (15)

Table 5. (Continued)

Comp. No.	^{1}H-NMR; δ (ppm)	MS (70 eV): *m/z* (%)
3 k	7.93 (s, 1 H, 4-H), 7.77 (s, 1 H, 2-H), 7.55 (d, 2 H, ^3J = 8.6 Hz, 4-CH_3OC_6-*2′,6′-H*), 7.49 (s, 1 H, 9-H), 7.06 (d, 2 H, ^3J = 8.6 Hz, 4-CH_3OC_6-*3′,5′-H*), 6.28 (s, 1 H, 6-H), 3.88 (s, 3 H, CH_3O), 2.51 (s, 3 H, 5-CH_3)[a]	306 (100, M^+), 291 (14), 278 (17), 263 (20), 235 (6), 178 (8), 139 (9), 117 (10)
3 l	7.78 (s, 2 H, 2-H, 4-H), 7.55 (d, 2 H, ^3J = 8.5 Hz, 4-CH_3OC_6-*2′,6′-H*), 7.05 (d, 2 H, ^3J = 8.5 Hz, 4-CH_3OC_6-*3′,5′-H*), 6.27 (s, 1 H, 6-H), 3.88 (s, 3 H, CH_3O), 2.63 (s, 3 H, 9-CH_3), 2.50 (s, 3 H, 5-CH_3)[a]	320 (100, M^+), 305 (12), 292 (17), 277 (15), 178 (5), 146 (7), 124 (8)
3 m	7.98 (s, 1 H, 2-H), 7.81 (d, 1 H, ^3J = 9.6 Hz, 5-H), 7.80 (s, 1 H, 4-H), 7.50 (s, 1 H, 9-H), 7.17 (d, 1 H, ^4J = 3.0 Hz, 2,5-$(CH_3O)_2C_6$-*6′-H*), 6.99 (d, 1 H, ^3J = 8.9 Hz, 2,5-$(CH_3O)_2C_6$-*3′-H*), 6.91 (dd, 1 H, ^4J = 3.0 Hz, ^3J = 8.9 Hz, 2,5-$(CH_3O)_2C_6$-*4′-H*), 6.38 (d, 1 H, ^3J = 9.6 Hz, 6-H) 3.85 (s, 3 H, CH_3O), 3.83 (s, 3 H, CH_3O)[a]	322 (100, M^+), 307 (32), 279 (26), 208 (14), 161 (15), 148 (20), 136 (16), 125 (24), 76 (14), 63 (14)
3 n	7.94 (s, 1 H, 2-H), 7.90 (s, 1 H, 4-H), 7.49 (s, 1 H, 9-H), 7.15 (d, 1 H, ^4J = 2.9 Hz, 2,5-$(CH_3O)_2C_6$-*6′-H*), 7.15 (d, 1 H, ^3J = 8.9 Hz, 2,5-$(CH_3O)_2C_6$-*3′-H*), 6.93 (dd, 1 H, ^4J = 2.9 Hz, ^3J = 8.9 Hz, 2,5-$(CH_3O)_2C_6$-*4′-H*), 6.27 (s, 1 H, 6-H), 3.85 (s, 3 H, CH_3O), 3.83 (s, 3 H, CH_3O), 2.49 (s, 3 H, 5-CH_3)[a]	336 (100, M^+), 321 (26), 293 (22), 265 (10), 250 (9), 222 (9), 200 (10), 165 (10), 148 (19), 136 (10), 82 (6), 67 (7)
3 o	7.96 (s, 1 H, 2-H), 7.75 (s, 1 H, 4-H), 7.15 (d, 1 H, ^4J = 2.4 Hz, 2,5-$(CH_3O)_2C_6$-*6′-H*), 7.0 (d, 1 H, ^3J = 8.9 Hz, 2,5-$(CH_3O)_2C_6$-*3′-H*), 6.92 (dd, 1 H, ^4J = 2.4 Hz, ^3J = 8.9 Hz, 2,5-$(CH_3O)_2C_6$-*4′-H*), 6.26 (s, 1 H, 6-H), 3.84 (s, 3 H, CH_3O), 3.82 (s, 3 H, CH_3O), 2.64 (s, 3 H, 9-CH_3), 2.48 (s, 3 H, 5-CH_3)[a]	350 (100, M^+), 335 (29), 307 (19), 214 (21), 175 (15), 161 (16), 148 (44), 139 (21), 89 (13), 76 (16)

[a] $CDCl_3$

oxyphenyl) in position 3 and a different number of methyl groups in position 5 and 9 of the furocoumarin skeleton, were prepared. The synthesis of **1 c** [23], **2 a** [20], **2 b** [21], **2 c** [24], **2 g** [21], **2 h** [21], **2 j** [25], **3 a** [20], **3 b** [21], **3 c** [24], **3 g** [21], and **3 h** [21] has been described in the literature. All synthesized compounds have been characterized by ^{1}H-NMR- and mass spectroscopy and the new ones were further characterized by elemental analysis or high resolution mass spectroscopy.

Results and discussion

Absorption and fluorescence spectra

The absorption and fluorescence spectra of the potential photoreactive compounds were measured with the aim of characterizing their spectrophotometric- and fluorescence properties.

The maxima (λ_{max} (nm)) of the absorption spectra in the ground state of the synthesised furocoumarins (20–60 µM) dissolved in methanol are reported in Table 6. The large band shown in the spectra by **3 a** (292 nm) and **3 d** (296 nm) is shifted to longer wavelength if there is an aryl group in 3-position, e.g. **3 g** (298 nm), **3 j** (302 nm), and **3 m** (302 nm), instead of an alkyl group. In addition methyl groups in 5- and 9-position lead to a further bathochromic effect in the absorption spectra (**3 c**, **3 f**, **3 i**, **3 l**, **3 o**). All compounds exhibit sufficient absorption in the UVA wavelength range of 320–400 nm and in particular 350 nm, the maximum of the UVA lamp emission.

The maxima of the fluorescence excitation and emission spectra of the furocoumarins (4 µM) in methanol are reported in Table 6. The fluorescence excitation spectra show bands with maxima at around 300 or 330 nm. For compounds **3 a**–**i** the maxima in the fluorescence excita-

tion spectra agree with the absorption maxima, in the case of compounds **3 j**–**o** the spectra do not coincide. Former findings of Lysenko et al. [26, 27], measuring furocoumarins in ethanol, indicate that there exist monomeric and different aggregated forms of furocoumarins in solution. They suggested that only aggregated forms of furocoumarins possess the ability to fluoresce at a high degree, which may be responsible for their observed maxima discrepancy comparing the absorption and excitation spectra of different furocoumarins, which is similar to the observations in this work.

Lipophilicity

In a study on the biological action of furocoumarins, a relationship between the octanol/water partition coefficient (log *P*) and the structural changes resulting in increased affinities towards DNA was reported [28]. The variation of hydrophobicity seems to be a factor that affects the affinity of furocoumarins towards the internal lipophilic parts of duplex DNA [28–29]. For this reason the log *P* values were determined in order to characterize the lipophilicity of the synthesized furocoumarins. It is well established that reversed phase HPLC can provide quantitative information about the hydrophobicity of solute molecules, especially for highly hydrophobic compounds [30]. The log *P* values were obtained by a HPLC procedure [31], using an alkyl-bonded stationary phase and a methanol-water mixture as mobile phase. The compounds exhibited a wide range of log *P* values (2.23–4.69, Table 7). All furocoumarins showed a higher lipophilicity than 8-MOP. Comparing the furocoumarins in the series with each other, it can be observed that the introduction of a methyl group in 5-position (**3 b**, **3 e**, **3 h**, **3 k**, **3 n**) and in addition in 9-position (**3 c**, **3 f**, **3 i**, **3 l**, **3 o**) increases the lipophilicity. In the present studies no clear relationship between lipophilicity and DNA interaction could be found.

Interaction of furocoumarins with DNA

The photoreactivity of the 3-substituted compounds with nucleic acids was studied in order to investigate the influence of bulky and lipophilic substituents at the furan group on the ability to form crosslinks with DNA. Because the photoreactions are governed by preceding dark reactions, the interaction of furocoumarins with DNA in the dark was also examined.

As already described, three steps are generally assumed in the formation of furocoumarin-DNA photoadducts [2]. The first is a non-covalent interaction of the intercalation type. The non-covalent binding of the linear furocoumarins (**3 a**–**o**, 8-MOP, TMP) to DNA was investigated by fluorescence spectroscopy [28] and by measuring the thermal transitions of the DNA-macromolecule (melting experiments) [32].

It is well known that a preliminary complex in the ground state occurs between furocoumarins and DNA, in which the planar moiety of the furocoumarin undergoes intercalation inside DNA [2]. A furocoumarin in an interacting state should have a fluorescence maxima (λ_{max}) and a fluorescence intensity (I_f), which is different from that of the free state [33, 34]. The formation of a molecular complex between the compounds and DNA is supported by fluorescence quenching [28, 34, 35] and a shift of λ_{max} of the fluorescence excitation spectra of the small ligands. The recording of fluorescence excitation spectra instead of UV/VIS spectra, which have been used by other groups [13, 36, 37], has the advantage of lower furocoumarin concentrations in the aqueous medium. The fluorescence measurement data for the interaction of the synthesised furocoumarins and the reference compounds 8-MOP and TMP (4 µM) with calf-thymus DNA (600 µM) are shown in Table 6. The DNA titration experiments showed that the fluorescence intensity of the 3-methyl (**3 a**–**c**), and 3-*tert*-butyl derivatives (**3 d**–**e**) was quenched to a different extent with increasing DNA concentration, which is in accordance with previously reported furocoumarins [28, 34]. 8-MOP and TMP, which are known as DNA-intercalating compounds, showed a quenching of fluorescence intensity and a red shift of 9 nm in the fluorescence excitation spectra, when the compounds were dissolved in DNA-buffer solution instead of buffer solution alone. The 3-alkyl derivatives also showed a red shift, which is similar to that of the reference compounds, suggesting that the 3-methyl and 3-*tert*-butyl substituent may intercalate into DNA. The 3-aryl derivatives displayed a more different result. The 3-phenyl furocoumarins (**3 g**–**i**) showed a blue shift of 4–6 nm and a fluorescence quenching and the 3-(4-methoxyphenyl) compounds (**3 j**, **3 k**) showed red shifts and fluorescence quenching when the compounds were dissolved in DNA-buffer solution instead of buffer solution alone, suggesting that these compounds may intercalate in DNA. In the case of **3 l** and **3 o** no shift and quenching were observable, apparently due to only very weak or missing interactions between the compounds and DNA.

It has also been demonstrated that DNA shows helix stabilization when complexed with furocoumarins, leading to an increase in thermal helix to coil transition temperature (T_m increase) [29, 32]. The T_m (50 % of total thermal transition) increase could be measured from the melting profiles of poly(dA-dT)-poly(dA-dT) in the presence and absence of furocoumarin. The thermal denaturation data for interaction of the synthesized furocoumarins (**3 a**–**o**) and the reference compounds 8-MOP and TMP are

Table 6. Absorption and fluorescence properties of furocoumarins **3** shown in methanol, and in buffer pH 7.4 without or in presence of DNA.

Compound	λ_{max} (nm); log ε MeOH[a]	λ_{max} (nm) MeOH[b] ex/em	λ_{max} (nm) buffer[c] ex/em	λ_{max} (nm) buffer, DNA[d] ex/em	I_b/I_f[e]	Δ λ_{max} shift (nm)[f] ex
3 a	292; 4.00	292/450	299/478	310/474	0.9	11[g]
3 b	292; 4.00	292/436	298/460	310/460	0.6	12[g]
3 c	298; 4.05	298/445	304/466	311/466	0.4	7[g]
3 d	296; 4.05	296/445	302/475	310/475	0.8	8[g]
3 e	292; 4.08	292/430	299/458	309/458	0.7	10[g]
3 f	298; 3.91	298/439	308/432	314/432	1.0	6[g]
3 g	298; 4.10	298/468	341/454	337/454	0.9	4[h]
3 h	296; 4.08	296/456	333/450	327/450	0.9	6[h]
3 i	300; 4.03	300/456	324/417	318/450	0.5	6[h]
3 j	302; 4.08	338/450	331/456	341/456	0.8	10[g]
3 k	302; 3.97	333/454	331/442	337/442	0.8	6[g]
3 l	304; 4.12	333/467	323/442	323/442	1.0	0[g]
3 m	302; 4.17	342/442	n.d.	n.d.	n.d.	n.d.
3 n	302; 3.97	334/442	n.d.	n.d.	n.d.	n.d.
3 o	306; 4.10	334/467	329/447	329/442	1.1	0[g]
8-MOP	302; 4.02	302/476	302/508	311/503	0.9	9[g]
TMP	296; 4.01	296/442	302/464	311/464	0.5	9[g]

[a] Absorption wavelength maxima (λ_{max}) in MeOH (scanned wavelength range: from 280 to 400 nm) and log ε (logarithm of molar extinction).
[b] Fluorescence excitation and emission wavelength maxima (λ_{max}) in MeOH; slits 15 and 20 nm.
[c] Excitation and emission wavelength maxima (λ_{max}) in buffer pH 7.4; slits 15 and 20 nm.
[d] Excitation and emission wavelength maxima (λ_{max}) in DNA-buffer solution pH 7.4 (600 µM); slits 15 and 20 nm.
[e] Quotient of fluorescence intensities of furocoumarins in DNA-buffer solution pH 7.4 (600 µM; I_b) and free in buffer pH 7.4 (I_f).
[f] Shift of maximum wavelength of fluorescence excitation spectrum in DNA-buffer solution pH 7.4 (600 µM) in relation to buffer pH 7.4.
[g] Red shift. h Blue shift. N.d. means not determined.

shown in Table 7. The results show that for some furocoumarins (**3 a**–**c**, **3 f**–**j**, 8-MOP, and TMP) an increase in T_m is observed, although only to a small extend in comparison with the shifts noticed for some compounds under UVA irradiation conditions. The furocoumarins (**3 d**–**e**, **3 k**–**o**) show no marked increase in T_m values. The data of the fluorescence measurement and the melting experiments support the formation of molecular complexes between most of the furocoumarins and DNA and indicate the possible intercalation of these ligands inside duplex DNA. In the case of the 3-aryl substituted compounds (**3 l**, **3 o**) the results indicate no DNA-interaction. Evidence for intercalation of these compounds can be obtained from DNA melting studies under irradiation. Crosslinking requires that the furocoumarin penetrates into the interior of the double helix [1, 2].

After intercalation of the furocoumarins in the dark the second step of the formation of furocoumarin-DNA photoadducts is the formation of photomonoadducts upon UVA irradiation of the non-covalent complex. The formation usually involves the 5,6 double bond of a pyrimidine and the double bond of either the furan-side or the pyrone-side of the furocoumarin [1, 2]. In the last step the furocoumarin linked by the furan-side can absorb a second photon and, if the geometrical arrangement is favorable, an interstrand crosslink with a pyrimidine belonging to the opposite DNA strand can be formed (Figure 1).

In order to investigate the potential of compounds for interstrand crosslink formation, melting studies under 60 min irradiation conditions at a 10 : 1 molar ratio of calf-thymus DNA and furocoumarin and under 30 min

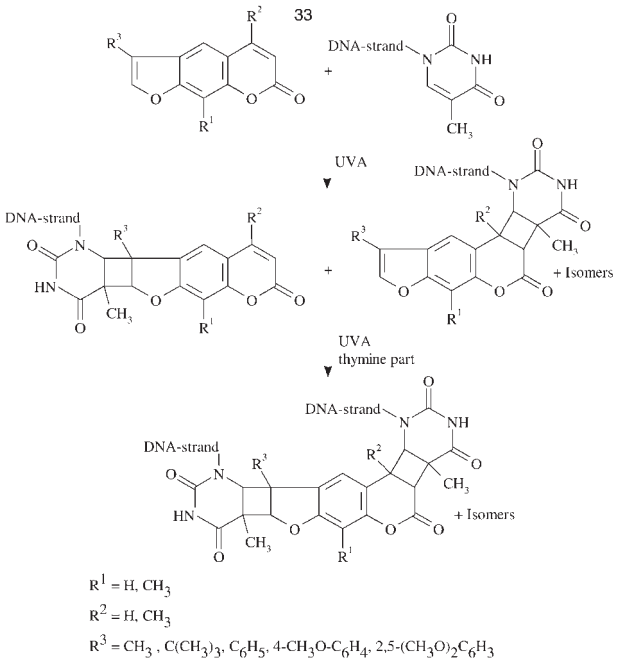


Figure 1. Possible photochemical formation of 2,3- and 5,6-monoadducts and 2,3,5,6-interstrand crosslink adducts (according to Song et al. [35] with modifications).

irradiation conditions at a 1 : 1 molar ratio of poly(dA-dT)-poly(dA-dT) and furocoumarin were made. The thermal denaturation data for interaction of the synthesized furocoumarins (**3 a**–**o**) and the reference compounds 8-MOP and TMP under UVA irradiation conditions are shown in Table 7.

In the calf-thymus DNA studies the greatest increase in Tm was induced by compound **3 c**. For TMP and the following furocoumarins it was gradually lower (**3 c** > TMP ⩾ **3 b** > **3 i** > **3 h** > **3 a** ~ 8-MOP > **3 g**). The other compounds (**3 d**–**f**, **3 j**–**o**) show no marked increase in T_m values. The amount of compound covalently bound to DNA generally increases with the A-T content [38]. For this reason it was expected, that there would be an increase in T_m values, when poly(dA-dT)-poly(dA-dT) was used instead of calf-thymus DNA. In this study compounds **3 a**–**c** and **3 g**–**j** also induce stabilization of the duplex DNA (**3 b** ~ **3 c** ~ TMP ⩾ **3 a** ~ **3 i** > 8-MOP ~ **3 g** > **3 h** ⩾ **3 j**). Compounds **3 d**–**f** and **3 k**–**o** exhibited no marked effect on T_m through UVA irradiation, which suggests, in addition to the results of the calf-thymus-DNA studies, that these compounds do not produce photoadducts or cross-links with DNA. Comparing the five series of furocoumarins with each other, it could be observed that the 3-methyl (**3 a**–**c**) and 3-phenyl (**3 g**–**i**) derivatives induced intense stabilization of poly(dA-dT)-poly(dA-dT) and the calf-thymus DNA helix and consequently marked photobinding, suggesting that these compounds are able to generate crosslinks similar to the reference compounds TMP and 8-MOP. Compounds containing a 3-*tert*-butyl (**3 d**–**f**), or 3-(4-methoxyphenyl) (**3 j**–**l**), or 3-(2,5-dimethoxyphenyl) (**3 m**–**o**) substituent exhibited no marked effect on T_m, suggesting that these compounds do not produce any photoadducts with poly(dA-dT)-poly(dA-dT) or calf thymus DNA.

The photoreaction of furocoumarins with DNA in general is influenced by several factors [38–40], e.g. the complex formation and geometrical arrangement of a furocoumarin after intercalation. Further investigations might be necessary to fully explain the present results, nevertheless some suggestion can be made: In comparison with the 3-methyl derivatives (**3 a**–**c**) a possible reason for the decreased (**3 g**–**i**) or missing (**3 d**–**f** and **3 j**–**o**) capacity to generate crosslinks with DNA might be a steric hindrance of the bulky 3-position, which may influence the geometrical arrangement of the furocoumarin in the DNA helix before irradiation with UVA light. In addition, specific furocoumarin excited states properties within the non-covalent complex might be important comparing the 3-phenyl derivatives (**3 g**–**i**) with the methoxyphenyl substituted compounds (**3 j**–**o**).

Photobleaching of *N,N*-dimethyl-*p*-nitrosoaniline (RNO)

Since PUVA is responsible for cell damage and the reaction with DNA, and proteins and lipids may also be influenced by the production of singlet oxygen [14], the capacity of synthesised compounds to photobleach RNO was evaluated in an *in vitro* test system [41], too.

Furocoumarins and UVA irradiation act through various mechanisms: a type I (radical formation), a type II (energy transfer mechanism, with formation of singlet oxygen) and the anoxic type III (C_4-cycloaddition reactions) [32]. The photobleaching capacity of the furocoumarins was studied according to the method of Krajic and El Moshni [41] which is based on the bleaching of *N,N*-dimethyl-*p*-nitrosoaniline by singlet oxygen in the presence of sensitizer and histidine (for more details see the experimental part). The results, reported in Table 7, show that all the furocoumarins are able to photobleach RNO, although with quite different yields. Generally the 3-alkyl substituted compounds (**3 a**–**f**) show a photobleaching capacity which is higher than that of the 3-aryl substituted ones (**3 g**–**o**). Some 3-aryl substituted furocoumarins have an ability to photobleach RNO which is similar to or lower than that of 8-MOP (**3 g**, **3 j**, **3 k**, **3 m**, **3 o**).

A consequence of the weak photobleaching capacity of the 3-aryl substituted compounds **3 g**, **3 j**, **3 k**, **3 m**, and **3 o** may be a decreased singlet oxygen generation capacity, similar to that of 8-MOP, which is known to be a

Table 7. T_m increase shown by DNA in presence of furocoumarins, photobleaching of RNO and log *P* values as measure of lipophilicity.

Compound	Induced ΔT_m shift[a] (°C) CT–DNA UV–A	Induced ΔT_m shift[b] (°C) poly–(dA–dT) dark	Induced ΔT_m shift[b] (°C) poly–(dA–dT) UV-A	Photo–bleaching of RNO[c] (%)	log *P*[d]
3a	2.0 ± 0.5[e]	0.5 ± 0.2[e]	5.1 ± 0.4[e]	24 ± 1.0	2.23 ± 0.09
3b	4.5 ± 0.5[e]	1.8 ± 0.2[e]	>15.0[e]	32 ± 1.0	2.69 ± 0.08
3c	8.5 ± 0.5[e]	1.3 ± 0.2[e]	>15.0[e]	34 ± 1.6	3.36 ± 0.06
3d	–0.3 ± 0.3	–0.1 ± 0.1	–0.5 ± 0.2	28 ± 1.0	3.42 ± 0.06
3e	0.2 ± 0.3	0.2 ± 0.2	–0.2 ± 0.3	37 ± 1.0	3.84 ± 0.05
3f	0.1 ± 0.3	0.7 ± 0.2[e]	–0.4 ± 0.3	20 ± 1.0	4.57 ± 0.05
3g	1.1 ± 0.3[e]	0.9 ± 0.2[e]	4.2 ± 0.2[e]	4 ± 1.0	3.26 ± 0.06
3h	2.3 ± 0.5[e]	0.5 ± 0.2[e]	3.0 ± 0.2[e]	10 ± 1.0	3.84 ± 0.05
3i	2.9 ± 0.5[e]	0.8 ± 0.3[e]	5.6 ± 0.5[e]	13 ± 1.0	4.57 ± 0.05
3j	0.1 ± 0.3	1.0 ± 0.1[e]	1.0 ± 0.4[e]	3 ± 0.7	3.51 ± 0.06
3k	–0.3 ± 0.3	0.3 ± 0.3	0.3 ± 0.2	7 ± 0.7	3.93 ± 0.05
3l	0.0 ± 0.3	0.1 ± 0.1	0.4 ± 0.2	9 ± 0.9	4.63 ± 0.05
3m	–0.4 ± 0.4	0.3 ± 0.2	0.3 ± 0.3	6 ± 1.5	3.51 ± 0.06
3n	0.1 ± 0.4	0.4 ± 0.3	0.0 ± 0.1	12 ± 1.4	3.93 ± 0.05
3o	–0.4 ± 0.4	0.3 ± 0.2	–0.1 ± 0.1	5 ± 1.0	4.69 ± 0.04
8–MOP	1.8 ± 0.5[e]	0.5 ± 0.2[e]	4.0 ± 0.2[e]	7 ± 0.6	1.69 ± 0.08
TMP	7.4 ± 0.5[e]	0.8 ± 0.1[e]	>15.0[e]	14 ± 1.0	3.36 ± 0.06
Methylene blue	n.d.	n.d.	n.d.	26 ± 1.0	n.d.

All values are mean ± s.e.m.

[a] Mean value from two determinations for a compound: calf-thymus (CT) DNA ratio of 1 : 10.

[b] Mean value from two to three determinations for a compound: poly (dA–dT) ratio of 1 : 1.

[c] Decrease in absorbance of *N,N*-dimethyl-*p*-nitrosoaniline (RNO) at 440 nm after 3 h of UVA irradiation. d Log *P* values as measure of lipophilicity determined by HPLC.

[e] Induced ΔT_m shift was significantly different with respect to control ($p < 0.05$).

poor singlet oxygen producer [15, 16], possibly resulting in a lower skin phototoxicity in comparison with the 3-alkyl substituted furocoumarins (**3a**–**f**). On the other hand, the 3-alkyl substituted furocoumarins (**3a**–**f**) may be the more photoreactive compounds, which may be interesting for developing new furocoumarins with specific target orientation.

Conclusions

The development of furocoumarins based on the introduction of bulky *tert*-butyl (**3d**–**f**), 4-methoxyphenyl (**3j**–**l**), and 2,5-dimethoxyphenyl (**3m**–**o**) substituents in the furan moiety of the planar compounds leads to novel derivatives which have, in comparison with 8-MOP and TMP, no crosslinking ability. This might be of interest in view of the side effects of furocoumarins in PUVA therapy. No clear relationship between lipophilicity and DNA interaction could be found, suggesting that steric effects, such as a steric hindrance generated by the bulky substituents at the 3-position, are responsible for this action. All novel derivatives are more lipophilic than 8-MOP and for this reason it may be expected that these compounds also associate with the relatively hydrophobic environment of lipid membranes or the hydrophobic pockets of proteins as further possible targets. The absorption and fluorescence spectra in methanol show that the investigated furocoumarins absorb UVA light and have fluorescent properties similar to those of other known furocoumarins. In addition, the different capacity of compounds to photobleach RNO, which might be correlated with the generation capacity of singlet oxygen, also influencing the reaction with DNA, proteins, and lipids, demonstrate that these furocoumarins may be interesting photoreacting agents, which should be investigated for further properties and other possible targets.

Acknowledgements

The author thanks Mrs Elke Stoll and Mrs Susan Häuer for technical assistance.

Experimental part

Chemistry

Melting points were determined on a Reichert Thermovar melting point apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker ARX 300 MHz FT NMR spectrometer, and chemical shifts (ppm) are reported relative to tetramethylsilane as internal standard in $CDCl_3$ or in DMSO-d_6 or the solvents peak ($CDCl_3$ 7.24 ppm, DMSO-d_6 2.5 ppm). Signals are designated as follows: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quadruplet), m (multiplet), ex (exchangeable with D_2O). Mass spectra were recorded on a Hewlett-Packard 5989A spectrometer (EI, 70 eV). High resolution mass spectra were recorded on a Finnigan MAT 8230 MS (70 eV), Institute for Organic Chemistry, University of Kiel. The ^{1}H-NMR and MS spectra of known compounds were in agreement with their chemical structure. Elemental analyses were performed by a Hewlett-Packard CHN autoanalyzer, Institute for Inorganic Chemistry, University of Kiel. Yields were not optimized. HPLC (Waters 590 pump, Waters 486 absorbance detector set at 210 nm or 310 nm) was performed on a LiChrospher 100 RP-18 column (5 µm particles; Merck). The absorbance spectra were recorded with a HP 8845A diode-array spectrophotometer fitted with a Julabo F20-C heating controller. The fluorescence spectra were obtained on a Perkin Elmer LS 50B luminescence spectrometer fitted with a Julabo F20-C heating controller and fused quartz cuvettes of 1 cm path length.

Compound **1 c** [23] was prepared according to the general procedure for preparation of coumarins [42]. Compounds **2 a** [20], **2 b** [21], **2 c** [24], **2 g** [21], **2 h** [21], and **2 j** [25] were prepared according to general procedure for preparation of β-ketoethers [43]. Compounds **3 a** [20], **3 b** [21], **3 c** [24], **3 g** [21], and **3 h** [21] were prepared according to general procedure for preparation of *7H*-furo[3,2-*g*]-1-benzopyran-7-ones [21].

General procedure for preparation of β-ketoethers [43]

The appropriate haloketone (6 mmol), potassium carbonate (2.8 g, 20 mmol) and 18-crown-6 (1 mL of stock solution (70 mg of 18-crown-6 in 100 mL of acetone)) were added to a solution of the required 7-hydroxy-2H-1-benzopyran-2-one **1** (5 mmol) in acetone (100 mL). The mixture was refluxed for 5 h (monitored by TLC). After cooling, the solution was filtered and evaporated under reduced pressure. The solid residue was recrystallized from ethanol.

The following compounds were prepared using the same general procedure for preparation of β-ketoethers [43].

7-(3,3-Dimethyl-2-oxobutoxy)-2H-1-benzopyran-2-one (**2 d**) was prepared from **1 a** (0.81 g, 5 mmol) and 1-bromo-3,3-dimethylbutan-2-one (1.07 g, 6 mmol).

4-Methyl-7-(3,3-dimethyl-2-oxobutoxy)-2H-1-benzopyran-2-one (**2 e**) was prepared from **1 b** (0.88 g, 5 mmol) and 1-bromo-3,3-dimethylbutan-2-one (1.07 g, 6 mmol).

4,8-Dimethyl-7-(3,3-dimethyl-2-oxo-butoxy)-2H-1-benzopyran-2-one (**2 f**) was prepared from **1 c** (0.95 g, 5 mmol) and 1-bromo-3,3-dimethylbutan-2-one (1.07 g, 6 mmol).

4,8-Dimethyl-7-(2-oxo-2-phenylethoxy)-2H-1-benzopyran-2-one (**2 i**) was prepared from **1 c** (0.95 g, 5 mmol) and 2-chloro-acetophenone (0.93 g, 6 mmol).

7-(2-(4-Methoxyphenyl)-2-oxoethoxy)-4-methyl-2H-1-benzopyran-2-one (**2 k**) was prepared from **1 b** (0.88 g, 5 mmol) and 2-bromo-4′-methoxyacetophenone (1.37 g, 6 mmol).

4,8-Dimethyl-7-(2-(4-methoxyphenyl)-2-oxoethoxy)-2H-1-benzopyran-2-one (**2 l**) was prepared from **1 c** (0.95 g, 5 mmol) and 2-bromo-4′-methoxyacetophenone (1.37 g, 6 mmol).

7-(2-(2,5-Dimethoxyphenyl)-2-oxoethoxy)-2H-1-benzopyran-2-one (**2 m**) was prepared from **1 a** (0.81 g, 5 mmol) and 2-bromo-2′,5′-dimethoxyacetophenone (1.55 g, 6 mmol).

7-(2-(2,5-Dimethoxyphenyl)-2-oxoethoxy)-4-methyl-2H-1-benzopyran-2-one (**2 n**) was prepared from **1 b** (0.88 g, 5 mmol) and 2-bromo-2′,5′-dimethoxy-acetophenone (1.55 g, 6 mmol).

7-(2-(2,5-Dimethoxyphenyl)-2-oxoethoxy)-4,8-dimethyl-2H-1-benzopyran-2-one (**2 o**) was prepared from **1 c** (0.95 g, 5 mmol) and 2-bromo-2′,5′-dimethoxyacetophenone (1.55 g, 6 mmol).

General procedure for preparation of 7H-furo[3,2-g]-1-benzopyran-7-ones [21]

The required 7-(2-alkyl-2-oxoethoxy)-2*H*-1-benzopyran-2-ones or 7-(2-aryl-2-oxoethoxy)-2*H*-1-benzopyran-2-ones (3 mmol) were heated in potassium hydroxide (1 N) solution (300 mL) under reflux in an atmosphere of nitrogen for 8 h. The solution was cooled, acidified with 30 % phosphoric acid, and kept at 4 °C overnight. The precipitate was collected by filtration, washed with water, dried, and recrystallized from ethanol (modified after MacLeod [21]).

The following compounds were prepared using the same general procedure for preparation of 7*H*-furo[3,2-*g*]-1-benzopyran-7-ones [21].

3-(1,1-Dimethylethyl)-7H-furo[3,2-g]-1-benzopyran-7-one (**3 d**) was prepared from **2 d** (0.78 g, 3 mmol).

3-(1,1-Dimethylethyl)-5-methyl-7H-furo[3,2-g]-1-benzopyran-7-one (**3 e**) was prepared from **2 e** (0.82 g, 3 mmol).

5,9-Dimethyl-3-(1,1-dimethylethyl)-7H-furo[3,2-g]-1-benzopyran-7-one (**3 f**) was prepared from **2 f** (0.87 g, 3 mmol).

5,9-Dimethyl-3-phenyl-7H-furo[3,2-g]-1-benzopyran-7-one (**3 i**) was prepared from **2 i** (0.93 g, 3 mmol).

3-(4-Methoxyphenyl)-7H-furo[3,2-g]-1-benzopyran-7-one (**3 j**) was prepared from **2 j** (0.93 g, 3 mmol).

3-(4-Methoxyphenyl)-5-methyl-7H-furo[3,2-g]-1-benzopyran-7-one (**3 k**) was prepared from **2 k** (0.97 g, 3 mmol).

5,9-Dimethyl-3-(4-methoxyphenyl)-7H-furo[3,2-g]-1-benzopyran-7-one (**3 l**) was prepared from **2 l** (1.02 g, 3 mmol).

3-(2,5-Dimethoxyphenyl)-7H-furo[3,2-g]-1-benzopyran-7-one (**3 m**) was prepared from **2 m** (1.02 g, 3 mmol).

3-(2,5-Dimethoxyphenyl)-5-methyl-7H-furo[3,2-g]-1-benzopyran-7-one (**3 n**) was prepared from **2 n** (1.06 g, 3 mmol).

3-(2,5-Dimethoxyphenyl)-5,9-dimethyl-7H-furo[3,2-g]-1-benzopyran-7-one (**3 o**) was prepared from **2 o** (1.11 g, 3 mmol).

Biological assay methods

Calf-thymus DNA, DMSO, NaCl, histidine, methylbenzene, ethylbenzene, propylbenzene, butylbenzene, pentylbenzene, and methanol Lichrosolv were purchased from Merck. Tris(hydroxymethyl)aminomethane (Tris), 2-(*N*-morpholino)propanesulfonic acid (MOPS), poly(dA-dT)-poly(dA-dT), *N,N*-dimethyl-*p*-nitrosoaniline, methylene blue, and 4,5′,8-trimethylpsoralen were purchased from Sigma. 8-Methoxypsoralen was purchased from Aldrich.

UV/VIS and fluorescence excitation and emission spectra of furocoumarins

The absorbance spectra were recorded using working solutions containing 20 to 60 μM of the furocoumarin in methanol (20 °C).

The fluorescence excitation and emission spectra of furocoumarins were studied using working solutions containing 4 μM of the furocoumarin in methanol (20 °C). The excitation and emission monochromators were set at the excitation or emission maxima of the furocoumarin solutions in methanol. The slit for the excitation monochromator was set at 15 nm and the slit for the emission monochromator was set at 20 nm.

Intrinsic fluorescence and dark binding of furocoumarins to DNA

An aqueous DNA stock solution was prepared by dissolving calf-thymus DNA (1.2 mM) in Tris-buffer (0.05 M; pH 7.4, 25 °C) containing 5 mM NaCl; working solutions contained 10–600 μM DNA. Drug and DNA-drug solutions were prepared by addition of the compounds from a stock solution in DMSO (400 μM) to give a final drug concentration of 4 μM. The solutions were incubated for 15 min in the dark. The maxima and the isosbestic points for furocoumarin and DNA-furocoumarin complex, and fluorescence excitation and emission spectra were determined. The interaction of furocoumarins with DNA was investigated by monitoring their intrinsic fluorescence at the isosbestic points in the free and bound (DNA-furocoumarin complex) states and in addition by recording the fluorescence excitation and emission spectra. The slit for the excitation monochromator was set at 15 nm and the slit for the emission monochromator was set at 20 nm.

UVA irradiation

Irradiation was performed by means of a 30-W Philips TL 36 P25/07 N lamp, wavelength range 320–420 nm, maximal emission at 350 nm; output measured at 360 nm with a Holtkamp UVA meter 360, was 2.5 and 0.9 mW/cm^2 at a distance of 13 and 26 cm, respectively.

Thermal transition studies

Absorbance-temperature profiles were determined. The temperature was directly measured in the cuvettes with a digital thermometer. Heating was applied at a rate of 1° min^{-1} with absorbance (260 nm) and temperature data sampling at 1 min intervals. DNA helix to coil transition temperatures (T_m) were determined at the midpoint of the melting profiles. Furocoumarin induced alterations in the DNA melting behavior are given by: $\Delta T_m = T_m$ (DNA + furocoumarin) – T_m (DNA); results are given as the mean of two to three determinations. Calf-thymus DNA and poly(dA-dT)-poly(dA-dT)-DNA were used as DNA compounds.

Calf-thymus DNA: an aqueous DNA-stock solution was prepared by dissolving calf-thymus DNA (1.2 mM) in MOPS-buffer (0.1 M, pH 7.0); working solutions contained 100 μM DNA. DNA-furocoumarin solutions were prepared by addition of the compounds from a stock solution in DMSO (1 mM) to give a final drug concentration of 10 μM. The solutions were irradiated for 60 min (output measured at 360 nm at a distance of 26 cm: 0.9 mW/cm^2). The addition of 20 μL of DMSO and UVA irradiation for 60 min had no effect on the transition temperature of the DNA in the absence of **3**.

Poly(dA-dT)-poly(dA-dT)-DNA (according to a modified method of Wulff et al. [44]): an aqueous DNA-stock solution was prepared by dissolving 25 units of the poly(dA-dT)-poly(dA-dT)-DNA in 2.5 mL of MOPS-buffer (0.1 M, pH 7.0); working solutions contained 10 μM DNA (20 μL stock solution). DNA-furocoumarin solutions were prepared by addition of the compounds from a stock solution in DMSO (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. (output measured at 360 nm at a distance of 26 cm: 0.9 mW/cm^2). The addition of 20 μL of DMSO and UVA irradiation for 30 min had no effect on the transition temperature of the polynucleotide in the absence of **3**.

Determination of photobleaching of N,N-dimethyl-p-nitrosoaniline (RNO)

Photobleaching capacity was determined in aqueous solutions according to a modified method of Kraljic and El Moshni [41]. Solutions were prepared in phosphate buffer (0.07 M, pH 7.4) containing the furocoumarin (30 μM), *N,N*-dimethyl-*p*-nitrosoaniline (40 μM) and histidine (10 mM). The solutions were irradiated for increasing periods of time and *N,N*-dimethyl-*p*-nitrosoaniline bleaching was determined by absorbance at 440 nm. Results are given as the mean of three determinations after 3 h of UVA irradiation (output measured at 360 nm at a distance of 13 cm: 2.5 mW/cm^2). Control experiments were performed in the absence of histidine and showed that the tested furocoumarins were not able to bleach RNO directly, which is similar to the observations of Joshi and Pathak [15] who investigated 8-MOP and other furocoumarins. They carried out singlet oxygen quenching studies with azide ions and 1,4-diazabicyclo[2,2,2]octane to obtain additional evidence for singlet oxygen, and they also showed, that superoxide dismutase, which is a superoxide radical anion quencher, has no effect on the RNO bleaching test, suggesting that superoxide radical anion, which is produced by furocoumarins, too, does not influence the values of RNO bleaching [15, 16].

Log P determination

There is a linear relationship between the capacity factor log *k′* of compounds and their log *P* values [31]. The log *k′* values of five compounds (methylbenzene (0.04), ethylbenzene (0.19), propylbenzene (0.37), butylbenzene (0.56), and pentylbenzene (0.74)) with known log *P* values (2.69, 3.15, 3.68, 4.26, 4.82) [45] were determined as reference values. A plot of log *k′* versus log *P* generated from this calibration mixture was used for the calculation of log *P* values of furocoumarins, which log *k′* values were determined by the same procedure as for the reference compounds. Chromatographic retention data are expressed by the logarithm of the capacity factor log *k′*, defined as $\log (t_r - t_0)/t_0$, where t_r and t_0 are the retention times of the furocoumarin and of a non-retained compound (methanol). Solutions of the furocoumarins in methanol were injected into the chromatograph. Methanol/water/phosphoric acid (77/23/0.1) pH 3.1 was used as eluant (flow rate 1 mL/min). Because this reversed-phase HPLC procedure was also used as standard method for the determination of log *P* values of carbonic acids (data not published), a pH of 3.1 was set.

References

[1] J. A. Parrish, T. B. Fitzpatrick, L. Tannenbaum, M. A. Pathak, *N. Engl. J. Med.* **1974**, *291*, 1207–1211.

[2] D. Averbeck, *Photochem. Photobiol.* **1989**, *50*, 859–882.

[3] F. P. Gasparro, *Photochem. Photobiol.* **1996**, *63*, 553–557.

[4] R. L. Edelson, *Yale J. Biol. Med.* **1989**, *62*, 565–577.

[5] H. P. van Iperen, G. M. J. Beijersbergen van Henegouwen, *J. Photochem.Photobiol. B* **1997**, *39*, 99–101.

[6] O. Gia, A. Anselmo, M. T. Conconi, C. Antonello, E. Uriarte, S. Caffieri, *J. Med. Chem.* **1996**, *39*, 4489–4496.

[7] D. Averbeck, E. Moustacchi, *Photochem. Photobiol.* **1980**, *31*, 475–478.

[8] F. Dall'Acqua, D. Vidaldi, S. Caffierii, A. Guiotto, P. Rodighiero, F. Baccichetti, F. Carlassare, F. Bordin, *J. Med. Chem.* **1981**, *24*, 178–184.

[9] P. Rodighiero, M. Palumbo, S. Marciani Magno, P. Manzini, O. Gia, R. Piro, A. Guiotto, *J. Heterocycl. Chem.* **1986**, *23*, 1405–1410.

[10] F. Bordin, F. Carlassare, M. T. Conconi, A. Capozzi, F. Majone, A. Guiotto, F. Baccichetti, *Photochem. Photobiol.* **1992**, *55*, 221–229.

[11] A. Chilin, C. Marzano, A. Guiotto, P. Manzini, F. Baccichetti, F. Carlasse, F. Bordin, *J. Med. Chem.* **1999**, *42*, 2936–2945.

[12] J. Moron, C. H. Nguyen, E. Bisagni, *J. Chem. Soc. Perkin Trans. I,* **1983**, 225–229.

[13] C. Antonello, G. Zagato, S. Bobilio, C. Marzano, O. Gia, E. Uriarte, *Farmaco* **1994**, *49*, 277–280.

[14] Z. Zarebska, *J. Photochem. Photobiol. B.* **1994**, *23*, 101–109.

[15] P. C. Joshi, M. A. Pathak, *Biochem. Biophys. Res. Commun.* **1983**, *112*, 638–646.

[16] M. A. Pathak, P. C. Joshi, *Biochim. Biophys. Acta* **1984**, *798*, 115–126.

[17] D. Vedaldi, S. Frank, A. Pozzan, G. Miolo, F. Dall'Acqua, S. Caffieri, P. Rodighiero, A. Guiotto, *Farmaco* **1995**, *50*, 615–623.

[18] M. Ifster, P. Fossa, G. Menozzi, L. Mosti, F. Baccichetti, C. Marzano, M. Simonato, *Farmaco* **1995**, *50*, 669–678.

[19] F. Bordin, F. Baccichetti, C. Marzano, F. Carlassare, G. Miolo, A. Chilin, A. Guiotto, *Photochem. Photobiol.* **2000**, *71*, 254–262.

[20] J. N. Ray, S. S. Silooja, V. R. Vaid, *J. Chem. Soc.* **1935**, 813–816.

[21] J. K. MacLeod, B. R. Worth, R. J. Wells, *Aust. J. Chem.* **1978**, *31*, 1533–1544.

[22] V. N. Gupta, T. R. Seshadri, *J. Sci. Ind. Res.* **1935**, *16B*, 257–260.

[23] S. Ragaswami, T. R. Seshadri, *Proc. Indian Acad. Sci. Sect. A* **1937**, *5*, 112–114.

[24] D. R. Bender, J. E. Hearst, H. Rapoport, *J. Org. Chem.* **1979**, *44*, 2176–2180.

[25] Y.-L. Chen, T.-C. Wang, S.-C. Liang, C.-M. Teng, C.-C. Tzeng, *Chem. Pharm. Bull.* **1996**, *44*, 1591–1595.

[26] E. P. Lysenko, A. Y. Potapenko, V. L. Sukhorukov, *Biophysics.* **1988**, *33*, 804–807.

[27] E. P. Lysenko, V. L. Sukhorukov, Z. Vunderlikh, L. I. Shagova, G. A. Kuznetsova, L. V. Kuz'mina, A. Y. Potapenko, *Russ. J. Phys. Chem.* **1989**, *63*, 1518–1521.

[28] F. Dall'Acqua, D. Vedaldi. F. Bordin, F. Baccichetti, F. Carlassare, M. Tamaro, P. Rodighiero, G. Pastorini, A. Guiotto, G. Recchia, M. Cristofolini, *J. Med. Chem.* **1983**, *26*, 870–876.

[29] F. P. Gasparro, R. Dall' Amico, D. Goldminz, E. Simmons, D. Weingold, *Yale J. Biol. Med.* **1989**, *62*, 579–593.

[30] P. Vicini, E. Fisicaro, M. T. Lugari, *Arch. Pharm. Pharm. Med. Chem.* **2000**, *333*, 135–144.

[31] S. H. Unger, J. R. Cook, J. S. Hollenberg, *J. Pharm. Sci.* **1978**, *67*, 1364–1367.

[32] D. Vedaldi, S. Caffieri, S. Frank, F. Dall'Acqua, A. Jakobs, J. Piette, *Farmaco* **1995**, *50*, 527–536.

[33] M. Sasaki, I. Nakasato, H. Sugiura, H. Fujita, T. Sakata, *Photochem. Photobiol.* **1987**, *46*, 551–555.

[34] M. Sasaki, F. Meguro, E. Kumazawa, H. Fujita, H. Kakishima, T. Sakata, *Mutat. Res.* **1988**, *197*, 51–58.

[35] P.-S. Song, K. J. Tapley Jr., *Photochem. Photobiol.* **1979**, *29*, 1177–1197.

[36] V. Adam, M. Berger, J. Cadet, F. Dall′Acqua, B. Epe, S. Frank, D. Ramaiah, S. Raoul, C. Saha-Möller, D. Vedaldi, *Photochem. Photobiol.* **1996**, *63*, 768–778.

[37] M. Gupta, R. Ali, *J. Biochem.* **1984**, *95*, 1253–1257.

[38] A. Oroskar, G. Olack, M.J. Peak, F. P. Gasparro, *Photochem. Photobiol.* **1994**, *60*, 567–573.

[39] J. P. Demaret, S. Brunie, J. P. Ballini, P. Vigny, *Photochem. Photobiol.* **1989**, *50*, 7–21.

[40] F. Dall'Acqua, S. Marciani, G. Rodighiero, *Z. Naturforsch.* **1971**, *26b*, 561–569.

[41] I. Kraljic, S. A. El Moshni, *Photochem. Photobiol.* **1978**, *28*, 577–581.

[42] O. Dann, G. Illing, *Liebigs Ann. Chem.* **1957**, *605*, 158–167.

[43] S. Lam, E. Grushka, J. Chassin, *J. Anal. Chem.* **1978**, *50*, 1398–1399.

[44] H. Wulff, H. Rauer, T. Düring, C. Hanselmann, K. Ruff, A. Wrisch, S. Grissmer, W. Hänsel, *J. Med. Chem.* **1998**, *41*, 4542–4549.

[45] H. Kaup, Ph. D. Thesis, University of Kiel, **1996.**