1-Thiomethoxsalen and 1-Thiopsoralen: Synthesis, Photobiological Properties, and Site Specific Reaction of Thiopsoralens with DNA

Andreas E. Jakobs* and Jacques Piette

Laboratories of Heterocyclic Organic Chemistry (B6) and Microbiology (B23), University of Liège, 4000 Liège, Belgium

Received October 17, 1994[⊗]

The sulfur analogues of psoralen and 8-methoxypsoralen (8-MOP) in the pyrone moiety were synthesized and compared to the parent compounds in terms of photoreactivity with viral M13mp19 RF DNA. The damaged viral DNA was transfected into *Escherichia coli* and scored for infectivity toward Ca-treated wild-type *E. coli*. This allowed a comparative study of the sulfur and oxygen analogues to be made in terms of photoreactivity. Furthermore, the DNA sequence specificity for the formation of monoadducts and cross-links of the four analogues was determined with ^{32}P-labeled oligonucleotides containing thymidine in different sequences. The most site specific of the studied psoralens is 8-MOP, while 1-thiopsoralen is the most reactive analogue. This new thio analogue of psoralen leads to the efficient formation of monoadducts and cross-links in any pyrimidine–purine site.

Introduction

The naturally occurring psoralen (**8a**) and its 8-methoxy derivative **8b** (methoxsalen, 9-methoxyfuro[3,2-*g*][1]benzopyran-7-one; 8-MOP) are the photoactive drugs used in the treatment of numerous skin diseases[1,2] and in the recently developed extracorporal photochemotherapy.[3,4] Owing to their ability to intercalate into nucleic acids and photoreact with the 5,6 double bond of pyrimidines, psoralens can form either monoadducts or interstrand cross-links.[5] This leads to important biological effects such as the blockage of DNA replication and RNA transcription and the induction of mutations. Psoralens were used as structural probes of nucleic acids[6] and are now also recognized as virucidal agents especially against enveloped viruses like the herpes virus or the human immunodeficiency virus type-1 (HIV-1).[7] This property is now being investigated for the blood cell products purification.[8,9] Safety considerations for this application require very reactive psoralens.[10] Several new psoralens have been designed to enhance the photobinding properties, the most important being aminomethyl or hydroxymethyl derivatives[11] and more recently heteroanalogues containing endocyclic nitrogen,[12,13] sulfur,[14] and selenium atoms.[15,16] Most of the heteroanalogues containing sulfur and selenium have been shown to inactivate viral DNA much more efficiently than the natural furocoumarins psoralen and 8-MOP and thus show great promise in photochemotherapy.[17] The introduction of a sulfur atom in the pyrone ring of psoralen has been shown to produce the most dramatic enhancement of the photoreactivity of the psoralen system. Compared to the parent compound **8a**, 7*H*-thiopyrano[3,2-*g*][1]benzofuran-7-one (**8c**) has a 17-fold higher rate constant of the light-induced inhibition of the infectivity of M13mp19 RF DNA in wild-type *Escherichia coli*.

Up until now, only the unsubstituted psoralens containing sulfur in the pyrone ring have been reported. The introduction of substituents is assumed to further modify the reactivity of the psoralen system. For example, the introduction of a methoxy group is expected to induce a bathochromic shift and enhance the molar extinction coefficient of the thiopsoralen at 365 nm compared to psoralen. This paper reports on a systematic investigation of the sulfur analogues of psoralen and 8-MOP in the pyrone moiety. For this purpose, 1-thio-8-MOP (**8d**) has been synthesized and the natural psoralens **8a,b** and the newly synthesized thiopsoralens **8c,d** (Figure 1) have been evaluated in terms of reactivity with DNA and site specificity. The psoralens were photoreacted with naked M13mp19 RF DNA in the presence and absence of oxygen. The damaged viral DNA was transfected into *E. coli* and scored for infectivity towards Ca^{2+}-treated wild-type *E. coli*. This allowed a comparative study to be made in terms of photoreactivity with DNA and photodynamic effect. Furthermore, the site specificity of the photoreaction was assessed by use of palindromic ^{32}P-labeled DNA oligonucleotides, allowing to determine quantitatively the amounts of monoadducts and cross-links at the different reactive sites of DNA.

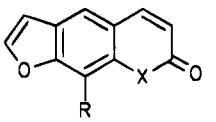

Figure 1. Structure of the psoralens and their sulfur analogues: **8a**, X = O, R = H; **8b**, X = O, R = OCH_3; **8c**, X = S, R = H; and **8d**, X = S, R = OCH_3.

Synthesis

In order to synthesize 9-methoxy-7*H*-thiopyrano[3,2-*f*][1]benzofuran-7-one (**8d**), the general method to obtain the unsubstituted heteropsoralens cannot be applied. Our strategy of synthesis (Figure 2) relies on the Newman–Kwart reaction to form the C(aromatic)–S bond in place of a phenol function.[18] The known 6,7-dimethoxy-5-benzo[*b*]furancarboxaldehyde (**2**) which is obtained in five steps from 1,2,3-trimethoxybenzene[19] (**1**) has been used as the starting mateial. The demethylation of the ether function in the *ortho* position of the aldehyde **2** can be performed by either aluminum chloride in 1,2-dichloroethane or lithium chloride in boiling dimethylacetamide. However, the later proce-

* Author to whom correspondence should be addressed. Laboratory of Heterocyclic Organic Chemistry (B6), University of Liège, 4000 Liège, Belgium.

⊗ Abstract published in *Advance ACS Abstracts*, February 1, 1995.

0022-2623/95/1838-0869$09.00/0

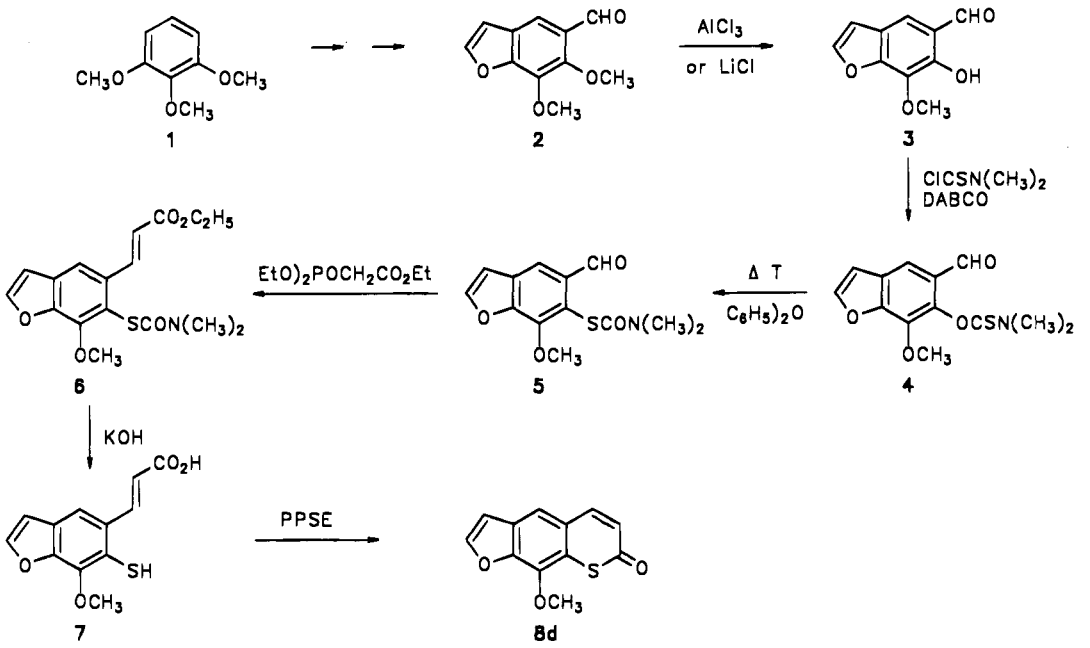

Figure 2. Synthesis of 1-thiomethoxsalen.

dure is more reproducible, and no tar is formed during the reaction making the workup easier. The so obtained phenol **3** is then reacted with (dimethylamino)thiocarbamoyl chloride to afford the carbamatethione **4** which is thermally rearranged in diphenyl ether to the thiocarbamate **5** with a 60% yield. To our knowledge, this is the first Newman–Kwart rearrangement involving an unprotected aldehyde function notwithstanding the reputation of this function to decompose under the Newman–Kwart reaction conditions.

The aldehyde function of the thiocarbamate **5** is then transformed into its vinylogous ester **6** by a Wittig–Horner olefination. Both the ester and thiocarbamate functions are hydrolyzed in one step with potassium hydroxide to the acid **7** which is cyclized with polyphosphoric acid silyl ether (PPSE) to provide 9-methoxy-7*H*-thiopyrano[3,2-*f*][1]benzofuran-7-one (**8d**).

Biological Evaluation

UV–Visible Spectra of the Heteropsoralens. The UV absorption spectra of the four psoralens **8a–d** are shown in Figure 3. The absorption band of the peak in the UVA region of 1-thio-8-MOP (**8d**) shows a bathochromic displacement of 20 nm and a hypsochromic shift compared to that of **8b**. At 365 nm, which is the wavelength used in photochemotherapy, the bathochromic shift more than compensates the hypsochromic shift and the molar extinction coefficient of 1-thio-8-MOP (**8d**) (ϵ = 1550 dm^3 mol cm^{-1}) is higher than that of the natural psoralens **8a** (ϵ = 400 dm^3 mol cm^{-1}) and **8b** (ϵ = 675 dm^3 mol cm^{-1}) but only about one-half that of thiopsoralen (**8c**) (ϵ = 2950 dm^3 mol cm^{-1}).

Inhibition of the Infectivity of M13mp19 RF DNA. The ability of the thiopsoralens to photoinactivate viral M13mp19 RF DNA is shown in Figure 4. The logarithm of the remaining fraction of intact M13mp19 RF DNA (log T_N/T_0) is presented as a function of the irradiation time for each tested psoralen. No loss of infectivity is observed in the absence of psoralens. The most important pathway involves [2 + 2] photoreactions of the intercalated psoralens with DNA. Nevertheless,

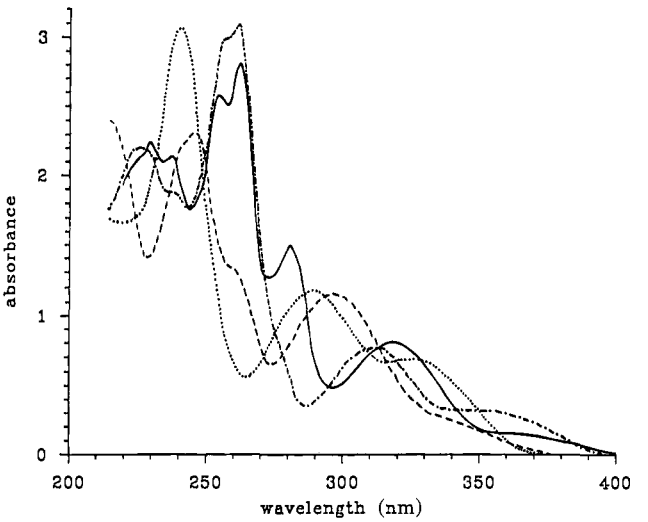

Figure 3. UV absorption spectra of the heteroanalogues **8a** (···), **8b** (– – –), **8c** (·–·–), and **8d** (—).

an oxygen-dependent photoinactivation pathway involving $^1O_2$ could also contribute to the induced photolesions. The later pathway is excluded when the photoinactivation reaction is conducted in oxygen-free solutions. The relative rate constants compared to that of psoralen (**8a**) are 1.1, 9.6, and 0.30 for compounds **8b–d**, respectively. Psoralen (**8a**) and methoxsalen (**8b**) have similar photoinactivating properties. Thiopsoralen (**8**) inactivates the infectivity of M13mp19 RF DNA much faster than the natural psoralens **8a,b** although to a lower rate than in the previously published results,[17] probably due to the use of a different light source. Surprisingly, the reactivity of 1-thiomethoxsalen (**8d**) is lower than that of the natural psoralens despite its enhanced light absorption at 365 nm.

In order to assess the importance of a possible interaction of the new heteropsoralen **8d** with molecular oxygen, which is in some cases responsible for the quenching of the excited state of intercalated psoralens, the photoinhibition reaction of M13mp19 RF DNA was repeated in the absence of molecular oxygen. The

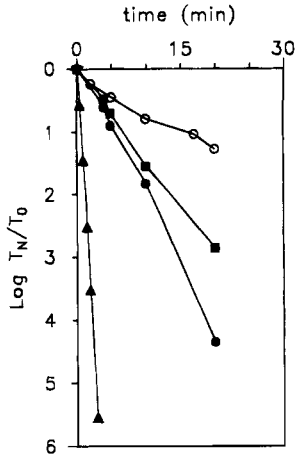

Figure 4. Inactivation of M13mp19 RF DNA by the heteropsoralens **8a**–**d** plotted as log T_N/T_0 vs irradiation time: **8a**, ■; **8b**, ●; **8c**, ▲; and **8d**, ○.

photoinactivating properties of 1-thiomethoxsalen (**8d**) were not modified in degassed solution, showing that activated oxygen species were not responsible for the loss of infectivity of M13mp19 RF DNA during irradiation with **8d**. Inversely, quenching of the excited state of **8d** by molecular oxygen can be excluded to explain the low photoinactivation properties of the intercalated **8d**. The photoinhibition of the infectivity of M13mp19 RF DNA by the four compounds **8a**–**d** could thus be attributed to their reactivity with the DNA bases.

Photoreaction with Oligonucleotides. Although the reactivity of the different psoralens with DNA depends to some extent on the surrounding sequence, the preferred reaction site for 8-MOP[20] (**8b**) and 4,5′,8-trimethylpsoralen has been shown to be the 5′-TpA sequence.[21,22] This site is also a hot spot for cross-link formation.[23] Small oligonucleotides could be used to assess the ability of the psoralens to form monoadducts and diadducts (Figure 5) in different base sequences. In order to measure the site specificity of the compounds **8a**–**d**, they were reacted with the synthetic ^{32}P-marked oligonucleotides *Kpn*I, *Bam*HI, and *Pst*I. The *Kpn*I linker [d(5′-CCGGTACCGG-3′)] which contains a central 5′-TpA sequence yielded both monoadducts and diadducts (Figure 5) with the four compounds tested. The order of formation of the monoadducts at this site is **8c** > **8a** > **8b** > **8d** (Figure 6), while that for the formation of the diadduct is **8c** ≫ **8b** > **8a** > **8d** (Figure 7). 8-MOP (**8b**) formed about 4 times more diadducts than psoralen (**8a**) but only one-half the amount of monoadducts. Since only the (furan side) monoadduct could give rise to the diadduct, it could be assumed that the monoadduct of 8-MOP (**8b**) was induced about twice as rapidly and was more easily converted to the diadduct than psoralen (**8a**). However, the photoinactivation of M13mp19 RF DNA proceeds at about the same rate with **8a**,**b**, and psoralen (**8a**) was suspected to react more easily at sites other than the 5′-TpA hot spot compared to 8-MOP (**8b**). The thio analogue **8c** had a very high initial cross-linking reaction rate with the 5′-TpA hot spot (Figure 5): the rapidly forming monoadduct disappeared to form the diadduct with high efficiency. After 5 min of irradiation, **8c** induced about 10 times more photoadducts than **8b** which corresponded to the high photoinactivation rate of M13mp19 RF DNA which could be accounted for by the ease of formation of both monoadducts and diadducts with the 5′-TpA site. The fraction of diadducts reached a maximum at about 15 min after which the amount of diadducts stabilized probably due to the photolysis or photodimerization of **8c**. Two types of monoadducts were observed with thiopsoralen (**8c**): one was induced rapidly and disappears after a short irradiation time yielding probably the diadduct, while the other is formed steadily over the whole reaction time (Figure 5). The cross-linked fraction stabilized after the rapidly forming monoadduct disappeared. This suggested to us that thiopsoralen (**8c**) was able to form two types of monoadducts with *Kpn*I, one of which at least evolves into the diadduct while the other which was induced more slowly could be either the result of the photoreversion of the diadduct or the reaction product at another reactive site of *Kpn*I. This assumption was corroborated by the fact that the second monoadduct was formed much more slowly with light having a longer wavelength (360 nm, data not shown).

For the methoxy derivative **8d**, the diadduct of *Kpn*I occurs about 20 times slower than the diadduct of 8-MOP (**8b**). This surprisingly low reactivity paralleled the low phototoxicity of thio-8-MOP (**8d**) vs M13mp19 RF DNA. Thiopsoralen (**8c**) reacted much faster than thio-8-MOP (**8d**), and consequently the introduction of the methoxy group reversed the order of reactivity from the oxygen to the sulfur series.

As the previous experiments suggested that psoralen (**8a**) reacted at sites other than the 5′-TpA sequence, the reactivity of the psoralens **8a**–**d** with the *Bam*HI linker [d(5′-CGGATCCG-3′)] which contained the 5′-ApT sequence was measured. Solely monoadducts were formed with psoralen (**8a**) and thio-8-methoxsalen (**8d**) which were attributed to the reaction with thymidine, while methoxsalen (**8b**) did not react with the 5′-ApT sequence in this linker under our reaction conditions. More surprisingly, thiopsoralen (**8c**) was able to form both a monoadduct and a cross-link with *Bam*HI although to a lesser extent than in the 5′-TpA site of *Kpn*I (Figure 6). In the *Pst*I sequence [d(5′-CCTG-CAGG-3′)], the same reaction pattern is observed for the four tested compounds. Thiopsoralen (**8c**) yielded both mono- and diadducts, suggesting that this compound easily forms diadducts of the thymidine–psoralen–cytosine type.

Discussion

The light absorption of the sulfur analogues of psoralen and 8-MOP was enhanced compared to that of the parent compounds. The introduction of the methoxy group in psoralen and thiopsoralen had a bathochromic effect which is accompanied by a hypsochromic effect in the oxygen series and a hyperchromic effect in the sulfur series. At 365 nm, thiomethoxsalen (**8d**) had a lower absorption coefficient than thiopsoralen but a higher absorption coefficient than the natural psoralens **8a**,**b**. However, a better light absorption does not necessarily induce a higher photoreactivity as shown by compound **8d**. The introduction of sulfur in place of oxygen was expected to induce a heavy atom effect, favoring the transition from the singlet excited state to the triplet state which is generally accepted to be responsible for the [2 + 2] photoreaction. The introduction of charge transfer groups (methoxy group) has been shown to modify the photophysical behavior of the

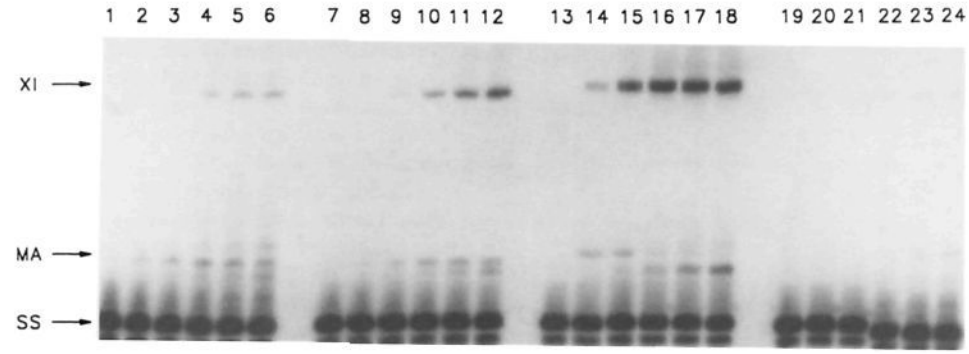

Figure 5. Analysis of the adducts induced into the oligonucleotide containing one *Kpn*I restriction site. Psoralen analogues were irradiated in the presence of the oligonucleotide as mentioned in the text. The various adducted oligonucleotides were separated by denaturating polyacrylamide gel electrophoresis. XL = cross-linked, MA = monoadducted, and SS = unreacted oligonucleotide. Lanes 1–6, **8a**; lanes 7–12, **8b**; lanes 13–18, **8c**; and lanes 19–24, **8d**; reaction times are 0, 2, 5, 15, 30, and 60 min, respectively.

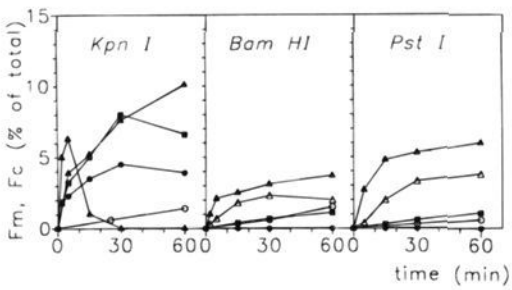

Figure 6. Kinetics of the formation of monoadducts (as percentage of total) with the oligonucleotides *Kpn*I, *Bam*HI, and *Pst*I vs irradiation time for **8a** (■), **8b** (●), **8c** (▲), and **8d** (○). For *Bam*HI and *Pst*I, the percentages of the cross-linked fractions with **8c** are indicated by hollow triangles.

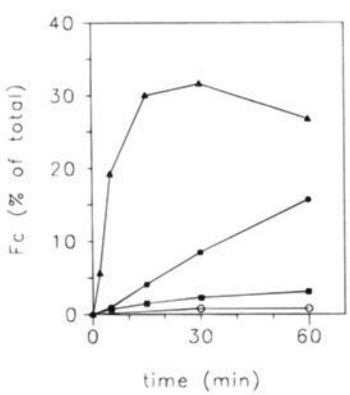

Figure 7. Kinetics of the formation of diadducts with the *Kpn*I oligonucleotide and the heteropsoralens **8a** (■), **8b** (●), **8c** (▲), and **8d** (○).

psoralens.[24,25] Furthermore, the introduction of sulfur in place of oxygen was expected to modify the geometry of the pyrone double bond. As the sulfur–carbon bond was longer than the oxygen–carbon bond, the pyronic double bond of the intercalated psoralen was probably nearer to the 5,6 double bond of thymidine, favoring the formation of the cross-link of the furan-side monoadduct.

The lower viral infectivity inhibition of thiomethoxsalen (**8d**) was due to a lower formation of both mono- and diadducts with thymidine. Thio-8-MOP (**8d**) was more stable in UV light than thiopsoralen (**8c**), and the possibility of rapid photoreversion of the monoadduct of **8d** could be excluded because the reaction rate of **8d** was not enhanced with light having a longer wavelength (data not shown). The lower cross-link yield of **8d** could not be attributed to a higher yield in the pyrone type adduct which was unable to yield the more lethal diadduct because the combined yield of the monoadducts was also lower with this compound. The poor photoreactivity of **8d** could be related either to its photophysical behavior or to sterical reasons occurring during the intercalation process. The methoxy group could modify the relative energy levels of the excited singlet states and thereby induce a "proximity effect" which favors a rapid radiationless transition[25] from the excited states (S_1 and S_2) to the ground state (S_0) and thus lower the quantum yield of the photoreaction.

On the other hand, sterical reasons have been shown to be important for the photochemical reactions of psoralens with DNA: the replacement of oxygen by sulfur combined with the introduction of the methoxy group (which points to the minor groove of DNA) problably hindered the proper intercalation of this compound in native DNA as well as in the studied oligonucleotides. The methoxy group strongly influences the site specificity of 8-MOP (**8b**) compared to psoralen **8a**: the former does not react with *Bam*HI and *Pst*I, whereas the later (**8a**) yields monoadducts with these sequences.

Concerning thiopsoralen (**8c**) which is able to form cross-links with any of the three studied linkers, it is far from certain that the reactive base is only thymidine. In the *Pst*I linker, a cross-link formed with the thymidine would result in the formation of a thymidine–cytosine cross-link. Psoralens have been shown to be able to react with cytosine although this is only a minor reaction of the furocoumarins. In the *Bam*HI oligonucleotide, however, such a cross-link is excluded because of the sequence and involvement of the thymidine would imply the formation of an unlikely T–G cross-link. Cross-link formation involving guanidine has never been described, and only one publication reported the very minor reaction of 8-MOP with adenosine.[26] It seems more likely to attribute the cross-link formation of the *Bam*HI site to an interstrand cytosine–psoralen–cytosine link since this is the only possibility to form cross-links with pyrimidines in opposite strands. In duplex DNA, other possibilities can be excluded for geometrical reasons. The formation of cytosine–HMT–cytosine cross-links has been described (*HMT: 4′-(hydroxymethyl)-4,5′,8-trimethylpsoralen*).[27] This implies that thiopsoralen (**8c**) is able to form cross-

links with any sequence containing pyrimidines to a significant amount since the yield of cross-link formation with cytosine is slightly higher than that of psoralen **8a** with the known hot spot 5′-TpA.

Whatever the reason for the poor photoreactivity of thio-8-MOP (**8d**), thiopsoralen (**8c**) seems to be the ideal compound to achieve the total photoinhibition of native DNA because of both its high photoreactivity and the ability to form interstrand cross-links in 5′-pyrimidine–purine sequences. This property should be particularly useful in the more recent applications of photoactive drugs. Further clinical studies are, however, required.

Conclusions

Preliminary studies had shown that psoralens could be used as virucidal agents for the blood product purification. More reactive compounds should warrant the safety of this application. We investigated the structure–activity relationship of the heteropsoralens containing sulfur in place of oxygen in the pyronic heterocycle. This has shown that the replacement of the pyronic oxygen atom by sulfur greatly enhanced the photobinding of the unsubstituted psoralen molecule but lowered the activity of the 8-methoxy-substituted psoralen. The enhanced inactivation of the infectivity of viral DNA of **8c** was due to a higher yield of both monoadducts and cross-links with DNA. Unlike the natural psoralens, **8c** efficiently induced DNA cross-links in a great number of different 5′-pyrimidine–purine sequences.

Experimental Section

General Synthetic Procedures. Unless otherwise stated reactions were carried out in commercial pure grade solvents without further purification. THF was distilled from sodium and potassium. Analytical grade anhydrous LiCl and pure grade KOH (minimum 85%; Janssen) were used. The standard isolation procedure consisted in pouring the reaction medium into ice/water mixture, filtering the precipitate, redissolving in CH_2Cl_2, washing with 1 N $NaHCO_3$, drying ($MgSO_4$), and evaporating the solvent under reduced pressure. Analytical thin-layer chromatography (TLC) was done with silica plates (Macherey-Nagel), and 70–230 mesh silica gel (E. Merck) was used for column chromatography. Melting points were determined with a Kofler hot plate melting point apparatus and are uncorrected. ^{1}H NMR spectra (in $CDCl_3$) are referenced to HMDSO (hexamethyldisiloxane), and coupling constants, J, are in hertz. ^{13}C NMR spectra are referenced to $CDCl_3$. Mass spectra were recorded by direct introduction under 70 eV IP. Elemental analyses were performed by the Institute of Pharmacy (Liege, Belgium).

6-Hydroxy-7-methoxybenzofuran-5-carboxaldehyde (3). A solution of 1.0 g (4.85 mmol) of 6,7-dimethoxybenzofuran-5-carboxaldehyde (**2**) in 10 mL of *N,N*-dimethylacetamide was heated to reflux under nitrogen. Lithium chloride (2.5 g, 59 mmol) was added, and heating was continued for 15 min. After cooling, the reaction mixture was poured into water and basified to pH 13 with 2 N NaOH. The solution was washed with ether (50 mL), and the aqueous layer was acidified to pH 1 with 6 N HCl and extracted twice with ether. The organic layer was washed with brine and dried and the solvent evaporated. Column chromatography (toluene/$CHCl_3$, 80/20) yielded 425 mg (46%) of **3**. The spectral data of **3** are in accordance with ref 19.

5-Formyl-6-[[(dimethylamino)thiocarbamoyl]oxy]-7-methoxybenzofuran (4). 6-Hydroxy-7-methoxybenzofuran-5-carboxaldehyde (**3**) (1.8 g, 9.37 mmol), 2.34 g (20.9 mmol) of DABCO (1,4-diazabicyclo[2.2.2]octane), and 2 g (16.2 mmol) of dimethylthiocarbamoyl chloride in 35 mL of DMF were heated to 80 °C for 1 h. The mixture was poured into 10N NaOH, and the precipitate was extracted with ether. The organic layer was washed with water, 1 N HCl, and brine and dried. Evaporation of the solvent left 2.46 g (91%) of **4**: mp 112–114 °C. An analytical sample melted at 115 °C (toluene/heptane). ^{1}H NMR (400 MHz): δ 10.04 (s, 1H), 7.81 (s, 1H), 7.67 (d, J = 2.0, 1H), 6.84 (d, J = 2.0, 1H), 4.19 (s, 3H), 3.48 (s, 3H), 3.44 (s, 3H). MS: m/z 297 (13), 234 (100), 207 (34), 206 (34), 191 (21), 175 (77). Anal. ($C_{13}H_{13}NO_4S$) C, H, N, S.

5-Formyl-6-[[(dimethylamino)carbonyl]thio]-7-methoxybenzofuran (5). **4** (2.8 g, 9.95 mmol) was added to 65 mL of diphenyl ether previously heated to 225 °C. Heating was continued for 40 min and the mixture allowed to cool. The reaction mixture was then column-chromatographed with a toluene–toluene/ethyl acetate (80/20) gradient to yield 1.73 g (62%) of **5**: mp 125 °C. ^{1}H NMR (400 MHz): δ 10.48 (s, 1H), 7.98 (s, 1H), 7.69 (d, J = 2.4, 1H), 6.86 (d, J = 2.4, 1H), 4.23 (s, 3H) 3.20 (s, 3H), 2.99 (s, 3H). MS: m/z 297 (10), 234 (43), 207 (23), 175 (28), 72 (100). Anal. ($C_{13}H_{13}NO_4S$) C, H, N, S.

5-[(Ethoxycarbonyl)vinyl]-6-[[(dimethylamino)carbonyl]thio]-7-methoxybenzofuran (6). 5-Formyl-6-[[(dimethylamino)carbonyl]thio]-7-methoxybenzofuran (**5**) (1.06 g, 3.03 mmol), 0.74 mL of triethylphosphonoacetate (3.73 mmol), 0.84 g of $Ba(OH)_2{\cdot}0.8H_2O$ (C-200), 0.153 mL (8.5 mmol) of water, and 35 mL of dry THF were refluxed for 1 h. The reaction mixture was poured into 10 N HCl and extracted with ether. The organic layer was washed with water and 1 N $NaHCO_3$ and dried. After evaporation of the solvent, the product was column-chromatographed (toluene–toluene/ethyl acetate, 80/20, gradient) to yield 1.0 g (75%) of **6**: mp 82 °C. ^{1}H NMR (400 MHz): δ 8.30 (d, J = 15.8, 1H), 7.63 (d, J = 2.1, 1H), 7.61 (s, 1H), 6.76 (d, J = 2.1), 6.35 (d, J = 15.8, 1H) 4.23 (q, J = 7.1, 2H), 4.21 (s, 3H), 3.1 (s, br, 6H) 1.31 (t, J = 7.1, 3H). MS: m/z 349 (50), 277 (57), 233 (16), 205 (57), 204 (72), 72 (100). Anal. ($C_{17}H_{19}NO_5S$) C, H, N, S.

5-(Carboxyvinyl)-6-mercapto-7-methoxybenzofuran (7). 5-[(Ethoxycarbonyl)vinyl]-6-[[(dimethylamino)carbonyl]thio]-7-methoxybenzofuran (**6**) (1.0 g, 2.86 mmol) and 2 g (35.7 mmol) of KOH were refluxed under nitrogen in 60 mL of aqueous ethanol (4/6, v/v) for 18 h. The reaction mixture was poured into water, acidified to pH 0 with 6 N HCl, and extracted with ether. The organic layer was washed with brine and dried. Evaporation of the solvent yielded 640 mg (89%) of **7** which was used without purification in the next step.

9-Methoxy-7*H*-thiopyrano[3,2-*f*][1]benzofuran-7-one (8d). P_4O_{10} (4.05 g, 14.3 mmol), 6.42 mL (30.2 mmol) of HMDSO, and 25 mL of $CHCl_3$ were refluxed until complete dissolution. The solvents were evaporated, and 100 mg (0.4 mmol) of the acid **7** was added. This mixture was heated to 160 °C for 1 h, allowed to cool, poured into iced water, and extracted with ether. The organic layer was washed with 1 N Na_2CO_3 and dried and the product column-chromatographed with toluene: yield, 30 mg (32%); mp 143 °C. ^{1}H NMR (400 MHz): δ 7.74 (d, J = 10.6, 1H), 7.67 (d, J = 2.0), 7.55 (s, 1H), 6.84 (d, J = 2.0), 6.47 (d, J = 10.6, 1H), 4.30 (s, 3H). MS: m/z 232 (100), 204 (77), 189 (100), 161 (17), 89 (32). Anal. ($C_{12}H_8O_3S$) C, H, S.

Chemicals, UV Spectra, Phages, Cell Cultures, and Oligonucleotides. Psoralen and 8-MOP were purchased from Sigma Chemicals and used without further purification. 7*H*-Thiopyrano[3,2–*f*][1]benzofuran-7-one was synthesized according to refs 15 and 16. UV spectra were recorded with a Perkin-Elmer 555 instrument in spectrometric grade methanol. Methods for the assessment of the photoreaction of the psoralen analogues with M13mp19 RF DNA purchased from Boehringer (Germany) have been described previously[17] except that a HBO-500 lamp (Osram) fitted with a WG 305 nm filter was used for the irradiations.

The palindromic oligonucleotides *Kpn*I, *Bam*HI, and *Pst*I were pourchased from Eurogentec (Belgium) and purified by electrophoresis on denaturating (7 M urea) 20% polyacrylamide gel. The bands were revealed by UV, cut and extracted overnight with 2 × 200 μL of water, dialyzed on a 2000 MW cutoff membrane against water at 4 °C, and concentrated on a Speedvac concentrator. The purified oligonucleotides were labeled with [γ-^{32}P]ATP (Boehringer Mannheim) with T4 polynucleotide kinase; 10 μg/mL final concentration of the labeled oligonucleotides was irradiated in 48 μL of TEN buffer

(10 mM Tris, 1 mM EDTA, NaCl 50 mM, pH 7.6) to which 2 μL of 3.75 mM ethanolic solution of the psoralens was added. The samples were irradiated at the same time at room temperature in adjacent quartz tubes with an incident light intensity of 400 kJ m^{-2} h^{-1} using a HBO-500 bulb (Osram) and a 305 WG filter unless specified otherwise. Aliquots (2.5 μL) were withdrawn after 0, 2, 5, 15, 30, and 60 min and electrophoresed on denaturating (7 M urea) 20% acrylamide sequencing gel with tracking dye containing bromophenol blue and xylene cyanol FF (2 μL) to resolve the nonreacted, monoadducted, and cross-linked oligonucleotides. The running buffer was 90 mM Tris borate, 2.5 mmol EDTA, pH 8.6. The fractions of molecules containing the different oligonucleotides were calculated after integration of LKB Ultrascan XL densitometry analysis of the autoradiograms and calculated as the percentage of total count in each lane.

Acknowledgment. J.P. is a Research Director supported by the National Fund for Scientific Research (NFSR, Brussels, Belgium). This work was supported in part by a NFSR research grant. We thank Professor A. Van de Vorst and Professor L. Christiaens for helpful discussions. Thanks is also due to Mr. T. Happaerts for his assistance in the synthesis of 1-thiomethoxsalen. We also thank the CREMAN for NMR spectra and the Institute of Pharmacy (Liège) for elemental analyses.

References

(1) Edelson, R.; Berger, C.; Gasparro, F.; Jegasoth, B.; Heald, P.; Wintroub, B.; Vonderhe, E.; Knobler, R.; Wolff, K.; Plewig, G. Treatment of cutaneous T-cell lymphoma by extra corporeal photochemotherapy. *N. Engl. J. Med.* **1987**, *316*, 297.
(2) Edelson, R. L. Photopheresis: present and future aspects. *J. Photochem. Photobiol. B: Biol.* **1991**, *10*, 165–174.
(3) Bisaccia, E.; Berger, C.; Dispaltro, F.; Armus, S.; Cahill, C.; Klainer, A. Viral-specific Immunization in AIDS-related Complex by Photopheresis. *Ann. N. Y. Acad. Sci.* **1991**, *636*, 321–330.
(4) Bisaccia, E.; Berger, C.; Klainer, A. Extracorporeal Photopheresis in the Treatment of AIDS-Related Complex: A Pilot Study. *Ann. Int. Med.* **1990**, *113*, 270–275.
(5) Tessman, J. W.; Isaacs, S. T.; Hearst, J. E. Photochemistry of the furan-Side 8-Methoxypsoralen-Thymidine Monoadduct Inside the DNA Helix. Conversion to Diadduct and to Pyrone-Side Monoadduct. *Biochemistry* **1985**, *24*, 1669–1676.
(6) Cimino, G. D.; Gamper, H. B.; Isaacs, S. T.; Hearst, J. E. Psoralens as Photoactive Probes of Nucleic Acid Structure and Function: Organic Chemistry, Photochemistry, and Biochemistry. *Annu. Rev. Biochem.* **1985**, *54*, 1151–1193.
(7) North, J.; Neyndorff, H.; Levy, J. G. Photosensitizers as virucidal agents. *J. Photochem. Photobiol. B: Biol.* **1993**, *17*, 99–108.
(8) Wisehahn, G. P.; Moore, P. A.; Lin, L.; Corash, L. The Use of Psoralen Compounds and Longwavelenght Ultraviolet Radiation for the Decontamination of Blood Products. *Photochem. Photobiol.* **1991**, *53* (Suppl.), 40.
(9) Goodrich, R. P.; Yerram, N. R.; Tay-Goodrich, B. H.; Forster, P.; Platz, M. S.; Kasturi, C.; Park, S. C.; Aebischer, J. N.; Rai, S.; Kulaga, L. Selective Inactivation of Viruses in the Presence of Human Platelets: UV Sensitization with Psoralen Derivatives. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 5552–5556.
(10) Fratantoni, J. C. Viral Inactivation of Blood Products: Regulatory Considerations. *Photochem. Photobiol.* **1991**, *53* (Suppl.), 58–59.
(11) Isaacs, S. T.; Shen, C. J.; Hearst, J. E.; Rapoport, H. Synthesis and Characterization of New Psoralen Derivatives with Superior Photoreactivity with DNA and RNA. *Biochemistry* **1977**, *16*, 1058–1064.
(12) Guiotto, A.; Chilin, A.; Pastorini, G.; Palumbo, M. Methylfuroquinolines: New Furocoumarin Isosters as Potential Photoreagents Toward DNA. *J. Heterocycl. Chem.* **1989**, *26*, 917–922.
(13) Quanten, E.; Adriaens, P.; De Schryver, F. C.; Roelandts, R.; Degreef, H. Photophysical Behaviour of new Pyrrolocoumarin Derivatives. *Photochem. Photobiol.* **1986**, *43*, 485–492.
(14) Wellman, G. R. Synthesis of 4,7,9-Trimethyl-2H-thieno[3,2-g]-1-benzopyran-2-one. A Psoralene Isostere. *J. Heterocycl. Chem.* **1980**, *17*, 911–912.
(15) Jakobs, A. E.; Christiaens, L. E.; Renson, M. J. Synthesis of Sulfur and Selenium Analogoues of Psoralen. *Heterocycles* **1992**, *34*, 1119–1132.
(16) Jakobs, A. E.; Christiaens, L. E.; Renson, M. J. Synthesis of Monosulphur and Monoselenium Analogues of Psoralen. *Tetrahedron* **1994**, *50*, 9315–9324.
(17) Jakobs, A.; Piette, J. Photobiological Activity of Sulphur and Selenium Analogues of Psoralen. *J. Photochem. Photobiol. B: Biol.* **1994**, *22*, 9–15.
(18) Newman, M. S.; Karnes, H. A. The Conversion of Phenols to Thiophenols via Dialkylthiocarbamates. *J. Org. Chem.* **1966**, *31*, 3980.
(19) Nore, P.; Honkanen, E. A New Synthesis of Methoxsalen. *J. Heterocycl. Chem.* **1980**, *17*, 985–987.
(20) Gia, O.; Magno, S. M.; Garbesi, A.; Colonna, F. P.; Palumbo, M. Sequence Specificity of Psoralen Photobinding to DNA: A Quantitative Approach. *Biochemistry* **1992**, *31*, 11818–11822.
(21) Esposito, F.; Brankamp, R. G.; Sinden, R. R. DNA Sequence specificity of 4,5′,8-Trimethylpsoralen Cross-linking. Effect of Neighboring Bases on Cross-linking the 5′-TA Dinucleotide. *J. Biol. Chem.* **1988**, *263*, 11466–11472.
(22) Ramaswamy, M.; Yeung, A. T. The Reactivity of 4,5′,8-Trimethylpsoralen with Oligonucleotides Containing AT Sites. *Biochemistry* **1994**, *33*, 5411–5413.
(23) Gamper, H.; Piette, J.; Hearst, J. E. Efficient Formation of a Crosslinkable HMT Monoadduct at the Kpn I Recognition Site. *Photochem. Photobiol.* **1984**, *40*, 29–34.
(24) Mantulin, W. W.; Song, P. S. Excited States of Skin-Sensitizing Coumarins and Psoralens. Spectroscopic Studies. *J. Am. Chem. Soc.* **1973**, *95*, 5122–5129.
(25) Lai, T.; Lim, B. T.; Lim, E. C. Photophysical properties of Biologically Important Molecules Related to Proximity Effects: Psoralens. *J. Am. Chem. Soc.* **1982**, *104*, 7631–7635.
(26) Ou, C. N.; Song, P. S. Photobinding of 8-Methoxypsoralen to Transfer RNA and 5-Fluorouracil-Enriched Transfer RNA. *Biochemistry* **1978**, *17*, 1054–1059.
(27) Boyer, V.; Moustacchi, E.; Sage, E. Sequence Specificity in Photoreaction of Various Psoralen Derivatives with DNA: Role in Biological Activity. *Biochemistry* **1988**, *27*, 3011–3018.

JM940684J