Phototherapy and Photopharmacology

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The activation of 8-methoxypsoralen (8-MOP) by long-wavelength ultraviolet A light (UVA, 320-400 nm) induces the formation of interstrand cross-links in DNA. Psoralen plus UVA (PUVA) is widely used in the treatment of psoriasis, a hyperproliferative disease of the skin. A new psoralen plus UVA therapy has been developed in which the 8-MOP-containing blood of cutaneous T cell lymphoma (CTCL) patients is irradiated with UVA light extracorporeally (i.e., extracorporeal photopheresis). The first group of patients had the leukemic variant of CTCL. A regimen of two treatments on successive days at monthly intervals produced a clinical response in eight of 11 patients. In this review the properties of several psoralens (both naturally occurring and synthetic derivatives) are compared, using several assays (DNA cross-linking, inhibition of lymphocyte response to mitogen stimulation, and cell viability). The development of a panel of monoclonal antibodies that recognize 8-MOP-modified DNA is also described. These antibodies have been used to quantitate 8-MOP photoadduct levels in human DNA samples. In addition to the psoralens, the light activation of two other compounds, gilvocarcin and an insulin-psoralen conjugate, is described.

"The sun is undoubtedly the best source of light; but as it is not always available, it is necessary to have recourse to artificial light, especially to electric light."

—from Phototherapy, Niels Finsen (1901)

INTRODUCTION

The birth of phototherapy occurred nearly 100 years ago. Niels Finsen had been treating lupus vulgaris (a cutaneous manifestation of tuberculosis) by heliotherapy, i.e., controlled exposure of his patients to sunlight. Given the fickle nature of sunlight, however, Finsen initiated a collaboration with an engineer who was a victim of lupus vulgaris and designed an artificial light source for the convenient treatment of patients [1]. This event marks the birth of modern phototherapy, for without easily manipulated and measured light sources the variety of today's phototherapies could not be administered. Finsen's pioneering efforts in the application of light, and artificial light in particular, earned him the Nobel Prize in Medicine in 1903. The development of artificial sources has continued to play an integral role in photomedicine.

The curative powers of sunlight were known in Biblical times. The solar spectrum of sunlight at the below-sea-level Dead Sea, filtered by passage through a thickened atmosphere, may have been responsible for the alleviation of many skin conditions [2]. The Egyptians ingested the leaves of a weed (ammi majus) that grew by the Nile to treat depigmented patches of skin [3]. The active ingredient of the ammi majus plant, 8-methoxypsoralen (8-MOP), was isolated and characterized in 1947 [4]. In the early

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1950s, Aaron Lerner became fascinated by 8-MOP, a naturally occurring, biologically inert substance which could be activated by sunlight to become a potent photosensitizer. A seminal publication [5] by Lerner and associates in 1953 established that purified 8-MOP could be administered safely to humans, was efficacious at low doses in the management of vitiligo, and raised the possibility that ultraviolet energy activates 8-MOP to an intermediary form responsible for the biologic potency of the parent molecule. That paper created great interest and initiated the modern era of therapeutic photopharmacology.

The mutagenic properties of 8-MOP were discovered by Musajo [6], who also later demonstrated that irradiated 8-MOP could react with DNA [7]. In 1970 it was suggested that the inhibition of the hyperproliferation of skin in psoriatic patients by photoactivated 8-MOP was secondary to its cross-linking of DNA [8].

All of the phototherapies that have been developed using 8-MOP thus far have involved the irradiation of skin after topical application or oral ingestion of the drug [9]. For the past four years we have been developing a new psoralen-based phototherapy in which the diseased blood of cutaneous T cell lymphoma patients is irradiated extracorporeally with ultraviolet A radiation (320–400 nm). The successful development of this new phototherapy depended on the creation of a convenient and efficient light source and an irradiation system for the extracorporeal treatment of human blood. This goal was achieved after collaboration with scientists at Johnson and Johnson, Inc.

In this review we describe the development of extracorporeal photopheresis. We also compare the properties of several natural and synthetic psoralens. Our long-term goal is to elucidate the details of psoralen photobiology and to apply the techniques of modern molecular biology to the development of more potent psoralens as well as other photopharmacologic agents.

**EXTRACORPOREAL PHOTOPHERESIS**

In recent years the physics, chemistry, and biology of light have been used to develop several light-mediated therapies (for a review see [10]). In 1974 an experimental photochemotherapy program using 8-MOP and UVA was initiated by Parrish et al. for the treatment of psoriasis, a hyperproliferative disease of the skin [11]. Healthy skin renews itself in an orderly fashion over approximately a one-month period. In psoriasis the renewal occurs in a disorderly fashion at an elevated rate, leading to the clinical signs of scaling and plaque formation [12]. The 8-MOP is taken orally two hours prior to irradiation of the skin with UVA light. In most cases the psoriasis can be cleared after approximately 20 treatments. More recently this form of therapy, referred to as PUVA, has been directed at the involved skin of patients with the epidermotropic neoplasm, cutaneous T cell lymphoma (CTCL) [13]. This is an effective therapy for the early stages of the disease; however, once beyond this stage PUVA is merely palliative [14].

To direct PUVA therapy more precisely at the involved blood of leukemic CTCL patients, Edelson et al. [15] have bypassed the skin and have developed a new phototherapeutic modality in which 8-MOP in the blood of patients is passed through a UVA exposure field prior to reinfusion. This form of therapy has several unique advantages. First, the lymphocytes are exposed to a biologically inert drug which is activated in situ by the flick of a switch. Second, due to the short lifetime of the photoactivated 8-MOP, all activity is lost by the time the blood is returned to the
patient, thus avoiding toxic side effects on other organs. Finally, non-nucleated blood components (red blood cells, platelets, and plasma proteins) are not significantly affected by this treatment.

Significant clinical responses were achieved with 12 treatments over a six-month period. This experimental therapy has now been in use for more than three years. The first group of patients had the leukemic variant of CTCL. These patients were selected because their disease was resistant to management with standard systemic therapy, their prognoses were poor, and their disease responded transiently to the removal of leukemic cells by leukapheresis. A conservative regimen of a cycle of two treatments on successive days at monthly intervals produced a clinical response in eight of 11 patients, as evidenced by decreased severity of skin lesions and laboratory responses—in particular, decreased numbers of abnormal T cells.

In designing and implementing an effective radiation system for the treatment of abnormal T cells, several experiments were performed to determine (a) which UVA wavelengths were most effective at inhibiting lymphocyte proliferation in the presence of 8-MOP; (b) the respective roles of monoadducts and cross-links; (c) what level of 8-MOP photoadduct formation occurred during photopheresis; and (d) what dose of UVA light was required to induce a clinical response.

**PSORALENS ARE PHOTOPHARMACOLOGIC AGENTS**

8-MOP is a naturally occurring tricyclic aromatic compound (Fig. 1), whose planar structure facilitates intercalation between nucleic acid base pairs. Activation of the intercalated complex with UVA light leads to photoadduct formation with pyrimidines, primarily thymines, in cellular DNA [16]. Three types of photoadducts can be formed: two monoaddition products and one diadduct or cross-link (Fig. 1). The distribution of these adducts in DNA is a function of the particular psoralen, the DNA conformation, and, perhaps, base sequence and the wavelength of irradiating light.

**ACTION SPECTRA STUDIES**

In our laboratory we have been elucidating the photochemistry and photobiology of psoralen photochemistry. We began our studies by determining which UVA wavelengths were most effective for the inhibition of mitogen-stimulated lymphocytes treated with 8-MOP [17]. Figure 2 shows that the optimal wavelength range (330–350 nm) is comparable to that found in other biological assays, as shown in Table 1. The optimal wavelength range does not include the absorption maximum for 8-MOP (303 nm) for two reasons. First, all of the assays essentially measure the ability of 8-MOP to potentiate wavelengths of UV light in a biological assay. UVB wavelengths (280–320 nm) alone have been shown to be potent inhibitors of biological functions; thus irradiation with wavelengths of light at which 8-MOP shows a strong absorption (i.e., 300–320 nm in the UVB range) does not result in a photoenhancing effect. Second, psoralens are known to form both mono- and di-adducts. The diadducts, or cross-links, form when a previously formed monoadduct absorbs a second UV photon. The 4',5'-monoadduct that is the precursor to the cross-link has an absorption spectrum that is shifted to longer wavelengths in comparison to that for 8-MOP (Fig. 3). Thus the formation of cross-links would be favored by irradiation with longer wavelengths of UVA light. To test this hypothesis, we developed a gel electrophoresis to measure psoralen cross-linking as a function of UVA wavelengths [18]. A homogenously sized sample of DNA was obtained by making a single blunt-end cut in circular DNA.
obtained from *E. coli*. Solutions of linear plasmid DNA (20 µg/ml) and 4'-aminomethyl-4,5',8-trimethylpsoralen (AMT) (50 ng/ml) were irradiated with sub-bands of UV light over the range 298–382 nm. The extent of cross-linking was determined by gel electrophoresis in 1 percent agarose gels. Prior to applying the samples to the gel, the samples in 0.2 N NaOH were thermally denatured (37°C for 15
Once in the running buffer, DNA molecules containing cross-links, which hold the base pairs in register, rapidly renature and migrate in the gel as double strands, while the molecules containing no adducts or monoadducts migrate as single strands (Fig. 4, upper panel). The number of double-stranded molecules and hence the number of cross-links is determined by densitometric scanning of the photographic negative of the ethidium bromide-stained gel (Fig. 4, lower panel). Analysis of cross-link formation at seven wavelengths showed that 320–350 nm light was optimal for cross-linking. Figure 5 shows that the action spectrum derived from these data correlates with the absorption spectrum of the 4',5'-monoadduct, indicating that this monoadduct is the precursor to the cross-link. This action spectrum also correlates with that for the inhibition of DNA synthesis shown in Fig. 2. These results support the

### TABLE 1
Optimal Wavelengths for 8-MOP Biological Assays

| Agent                        | Peak Activity |
|------------------------------|---------------|
| Human lymphocytes            | 334–346       |
| Human skin erythema          | 320–335       |
| Guinea pig skin erythema     | 330           |

*Note: See [17] and references therein.

**FIG. 3.** Absorption spectra of 8-MOP.  
(Upper panel): Psoralen monoadducts;  
(Lower panel): (---) 3,4-monoadduct;  
(-----) 4',5'-monoadduct.
FIG. 4. **Upper panel:** 1 percent agarose gel analysis of linear pBR322 irradiated with 346 nm light. The first three lanes contain known amounts of double helical linear DNA (20, 40, and 80 ng, respectively). Lane 4 is an unirradiated control (100 ng). Lanes 5–9 contain samples of DNA (100 ng each) exposed to increasing doses of light. **Lower panel:** Densitometric scan of the gel shown above. Dose of 346 nm light is indicated near each tracing.
contention of many workers that psoralen cross-links are responsible for the biological effects of PUVA therapies [19]. However, angelicins, which are angular isopсорalens incapable of cross-link formation, are also capable of inhibiting DNA synthesis, as described later.

Early in our studies we realized that it would be invaluable to be able to determine the in vivo levels of 8-MOP photoadduct formation in the DNA of lymphocytes treated with 8-MOP and UVA light. Scintillometry and HPLC were shown to be effective for the in vitro analysis of 8-MOP adduct formation in synthetic polynucleotides [20]. For example, HPLC methods were used to quantitate the yield of psoralen monoadducts as a function of irradiating wavelength and polynucleotide composition for samples in which the overall extent of modification was approximately 1 percent, a level easily achieved in vitro but much lower than that anticipated for the DNA obtained from the in vivo treatment of lymphocytes with 8-MOP and UVA light. Therefore we initiated a program for the production of a panel of monoclonal antibodies that would recognize 8-MOP photoadduct in DNA isolated from human lymphocytes.

**MONOCLONAL ANTIBODIES**

Balb c/Cr mice were immunized with 8-MOP-modified calf thymus DNA over a six-week period. Responsive mice were sacrificed and their spleens used to create hybridomas. The production and characterization of these antibodies were performed in collaboration with Dr. Santella of the Cancer Institute [21].

Figure 6 shows the results of competitive ELISA assays with a series of 8-MOP-modified polynucleotides. The 50 percent inhibition value for antibody 8G1 was 17 femtomoles of adduct in the original immuogen and 13 femtomoles in 8-MOP-modified poly(dA·dT) in which only monoadducts could form. The lower level of recognition in the alternating copolymer, poly(dAdT·dAdT), could be due to the presence of cross-links or an altered backbone conformation. These results suggest that this antibody primarily recognizes monoadducts. This hypothesis was verified by testing the individual adducts isolated by HPLC in a competitive assay.

These antibodies, produced by immunizing mice with modified calf thymus DNA, are capable of recognizing 8-MOP adducts in DNA isolated from human lymphocytes treated with 8-MOP and UVA. To calibrate these antibodies so that they could be used to assay nonradioactive samples from humans treated with 8-MOP and UVA, a series of lymphocyte samples were treated with tritiated 8-MOP and increasing doses of
UVA light. The DNA was isolated, and the overall extent of 8-MOP modification as a function of UVA dose was determined by liquid scintillometry (upper curve, Fig. 7). The same 8-MOP DNA samples were analyzed by a competitive ELISA assay using monoclonal antibody 8G1. A similar dose-related yield of adducts was observed (lower curve, Fig. 7). Since 8G1 has a high specificity for monoadducts [21], it would be expected to detect only a fraction of the adducts formed at the higher UVA doses as cross-links begin to accumulate [Santella RM, Gasparro FP; manuscript in preparation].

We have also used this antibody to quantitate 8-MOP adduct formation in the DNA isolated from human lymphocytes treated ex vivo with 8-MOP and UVA light (i.e.,

![Graph 6: Competitive ELISA assays for antibody 8G1. Competitors: o, 8-MOP-calf thymus DNA; o, 8-MOP-poly(dA-dT); ▼, 8-MOP-poly(dAdT-dAdT); ■, 4',5-dimethylangelicin-calf thymus DNA; and ▲, AMT-calf thymus DNA. Note: poly(dA-dT) is composed of the two homopoly nucleotides: poly(dA) and poly(dT). Poly(dAdT-dAdT) is a self-complementary copolymer.](image6.png)

![Graph 7: 8-MOP photoadduct formation in DNA isolated ex vivo with 8-MOP and UVA light. Upper curve: total 8-MOP incorporation as determined by liquid scintillometry analysis of tritium-labeled 8-MOP. Lower curve: fraction of total photoadducts recognized by monoclonal antibody 8G1 in a competitive assay for the same DNA samples analyzed by liquid scintillometry.](image7.png)
extracorporeal photopheresis. The isolated DNA samples were analyzed by competitive ELISA assays. The data shown in Table 2 represent the first quantitation of in vivo levels of 8-MOP modification of human DNA. The levels of modification range from 0.55 to 0.75 adducts per million bases. Thus a typical lymphocyte exposed to 8-MOP (~100 ng/ml) and UVA light (~1 J/cm²) could contain several thousand 8-MOP photoadducts. At this stage of our studies with this limited data, it is difficult to discern a correlation between the 8-MOP concentration in the blood and the ultimate level of adduct formation in the DNA. We have also used light microscopy in an indirect immunofluorescence assay to show that these antibodies recognize in situ 8-MOP photoadducts formed after UVA irradiation of skin to which 8-MOP had been applied topically [manuscript in preparation].

The repair, or lack of repair, of these adducts is ultimately responsible for the fate of a particular cell. Classical repair assays which can only measure overall repair and not the repair of specific adducts have indicated that both monoadducts and cross-links can be repaired [22]. Once a complete panel of monoclonal antibodies is available, i.e., at least one antibody specific for each photoadduct, we plan to study the repair kinetics for each. Furthermore, with improved sensitivity of the ELISA assays using either fluorescence detection or the biotin-avidin technology [23], the detection of unrepaired adducts should be feasible. DNA sequencing techniques will be used to discern the effects of sequence on the formation of psoralen photoadducts, their subsequent repair, and the location of unrepaired adducts in specific DNA sequences.

STRUCTURE-FUNCTION RELATIONSHIPS IN PSORALENS

The yield and distribution of psoralen photoadducts is strongly dependent on the number and nature of the substituents on the psoralen tricyclic ring system [24]. The sequential addition of methyl groups to the base molecule dramatically increases the binding constant ($K$) for intercalation between DNA base pairs. The data in Table 3 illustrate this phenomenon for angelicin and three of its methyl derivatives [25].

| TABLE 2 | 8-MOP Adduct Levels in DNA from Human Lymphocytes |
|---------|-----------------------------------------------|
| Patient | Free 8-MOP ng/ml | Bound 8-MOP per 10⁶ DNA Bases |
| TA      | 15              | 0.75                        |
| RO      | 25              | 0.55                        |
| DI      | 87              | 0.71                        |
| FI      | 13              | 0.69                        |

| TABLE 3 | Effect of Methyl Substituents$^a$ |
|---------|----------------------------------|
|         | Solubility ug/ml | $K$ 1/mole |
| Angelican (ANG) | 20 | 560 |
| MeANG       | 7  | 1,270 |
| Me₂ANG      | 5  | 3,760 |
| Me₃ANG      |    | 5,700 |

$^a$Averages for several synthetic angelicans; data from [25]
TABLE 4
Properties of Synthetic Psoralens

| Compound                  | Solubility μg/ml | K/10³ 1/mole | Intercalated Psoralens per 10⁴ Bases | % Inhibition* |
|---------------------------|-----------------|-------------|------------------------------------|--------------|
| 8-MOP                     | 38              | 0.77        | 390                                | 30           |
| AMT                       | 10⁴             | 152         | 50,000                              | 96           |
| 5-MOP                     | 5               | 2.8         | 1,400                               | —            |
| 4',5-Dimethyl-angelicin (DMA) | ~8⁴     | 1.5         | 700                                | 45           |

*Inhibition of lymphocyte proliferation assayed by tritiated thymidine incorporation after treatment with 10 ng/ml of the indicated psoralen and 3 J/cm² UVA light

*Data from [25]

FIG. 8. Comparison of synthetic psoralens to 8-MOP in the inhibition of lymphocyte proliferation. Lymphocytes (10⁶/ml) were irradiated (3 J/cm² UVA) in the presence of each psoralen at the indicated concentrations (for molecular structures of these compounds see [Berger CL, Cantor CR, Welsh J, Dervan P, Begley T, Grant S, Gasparro FP, Edeleon RL: Ann NY Acad Sciences 440:80-90, 1985]). Relative stimulation indices were computed by comparing the stimulation index in the presence of a test compound to that for a non-irradiated sample containing no test compound.
However, each additional methyl group makes the compound somewhat less water-soluble and thus less easily delivered as a therapeutic drug. To combat these problems, Isaacs et al. [26] synthesized a water-soluble form of 4,5',8-trimethylpsoralen (TMP) by substituting an amino methyl group at the 4' position of TMP, yielding 4'-aminomethyl-4,5',8-trimethylpsoralen, or AMT. Under physiological conditions the amino group carries a positive charge, thereby increasing its water solubility more than a thousandfold. Furthermore, the synergistic effects of the additional methyl groups and the positively charged amino group which can form a salt bridge with the phosphate moiety in the DNA backbone lead to a comparable increase in the intercalation binding constant. Thus, in comparison to 8-MOP under identical conditions, more than 100 times as much AMT can be intercalated between DNA base pairs (Table 4). This increased level of intercalation has an important effect. A higher extent of intercalation means that proportionately lower doses of light are required to form adducts. Furthermore, side reactions such as singlet oxygen formation or the formation of psoralen-psoralen photoadducts are minimized.

Two other psoralens, 5-MOP and TMP, have been used clinically [27]. We have been fortunate to obtain some synthetic psoralens as well as a series of psoralen analogs known as angelicins, which differ by virtue of their angular arrangement of the three-ring system. In an assay for the inhibition of lymphocyte proliferation, only one of the synthetic psoralens, AMT, showed a significantly greater activity than 8-MOP (Fig. 8). The potency of AMT is further demonstrated in Fig. 9 which shows that, with the same dose of UVA light, it can be activated at 20–50 times lower concentrations than 8-MOP. The particular effectiveness of AMT can be traced to the combined effects of the three methyl groups and the positively charged amino group which enhance the AMT-DNA binding constant. We have performed similar assays on several angelicins, which, owing to their geometry, cannot form cross-links. When their activity was compared to that for 8-MOP (Fig. 10) several were found to be comparable in potency, calling into question the long-held dogma that cross-links alone are responsible for the activity of psoralens. Rodighiero et al., from whom we received the angelicins, had shown previously that these compounds were clinically effective in the treatment of psoriasis [28]. In a series of papers, these authors have systematically demonstrated how the number of methyl substituents and their location can affect the photobiological activity of angelicins.
In addition to the composition of DNA, the reactivity of psoralens also depends on the nature of the psoralen substituents. Hearst et al. have shown that the number and location of methyl substituents on 8-MOP alters the yield and distribution of monoadducts in poly(dAdT) (Table 5). However, to date these studies have been performed on DNA in vitro. At present these are the only type of data that is available on psoralen reactivity toward nucleic acids. Under in vitro conditions in aqueous solutions, DNA exists in an extended double helical structure. Under in vivo conditions, however, as a result of DNA protein contacts, DNA is tremendously compacted as it is wound around proteins. The accompanying conformational changes can drastically alter the nature and availability of psoralen binding sites.

One of our goals is to use monoclonal antibodies as highly specific probes in combination with DNA sequencing methods to ascertain the location and identity of psoralen photoadducts in human DNA and to follow the repair or lack of repair of these adducts after extracorporeal photopheresis. The extension of these studies to other psoralens can lead to the rational development of more potent psoralen derivatives.

**TABLE 5**

| Compound          | 3,4-monoadduct | 4',5'-monoadduct |
|-------------------|----------------|-----------------|
| 8-MOP             | 19             | 75              |
| 4-methyl-8-MOP    | 2              | 94              |
| 5'-methyl-8-MOP   | 17             | 73              |
| 4,5'-dimethyl-8-MOP | 2          | 94              |

*Note: Data from [29]*
OTHER PHOTOACTIVATABLE DRUGS

We have also examined the potential therapeutic suitability of gilvocarcin, whose structure and absorption spectrum are shown in Fig. 11. Gilvocarcin is an antitumor antibiotic first isolated from streptomycetes [30]. The particular potency of this compound after photoactivation was first observed in a bacteriophage lysogenic assay [31]. While microgram quantities of this drug are capable of nicking DNA in the dark, we found that picogram quantities of gilvocarcin can be photoactivated with UVA or visible light (1–3 J/cm²) to induce the same level of single-strand breaks in superhelical DNA [32]. Figure 12 shows that gilvocarcin at concentrations 100 times lower than 8-MOP is a potent inhibitor of DNA synthesis in mitogen-stimulated lymphocytes after irradiation with long-wavelength UV light or visible light.

TARGETED DELIVERY OF PHOTOPHARMACOLOGIC AGENTS

The concept of using monoclonal antibodies to target toxic substances specifically to a specific cell population has been demonstrated in vitro [33]. However, the in vivo application of this technology is fraught with several difficulties. Chief among these is that humans treated with such antibodies may develop an immunologic response to the murine monoclonal antibodies to which the drug has been attached [34]. To circumvent this obstacle, we have been interested in the development of insulin, a species with relatively low immunogenicity, as a carrier molecule for photoactivatable substances. Murphy et al. [35] demonstrated that insulin is selectively bound and internalized by activated lymphocytes. We wished to take advantage of this specificity by covalently attaching a psoralen derivative to insulin and thus have created AMT-insulin [Yemul S, Knobler RM, Gasparro FP; unpublished data]. Gel electrophoresis analysis on 1 percent agarose gels, as described above, showed that this chimeric molecule retained its ability to cross-link DNA. Furthermore, when lymphocytes were treated with AMT-insulin, cell viability, as gauged by trypan blue exclusion, was selectively affected in those cells exposed to UVA light but not those kept in the dark (Fig. 13). Only stimulated cells take up insulin. The data in the left panel of Fig. 13 show that the
viability of lymphocytes stimulated with PHA was reduced over a five-day period, while unstimulated cells (right panel) were not affected.

Using another targeting strategy Yemul et al. have synthesized liposomes containing twofold specificity. Photoactivatable pyrene has been incorporated in the lipid bilayers. To direct the pyrene-containing liposomes to malignant T cells, T cell-specific monoclonal antibodies have been covalently attached to the liposome surface [36]. Thus the antibody-directed liposomes were selectively bound to a targeted subset of lymphocytes in the total human peripheral blood lymphocytes. After UVA irradiation, the T cells were selectively killed.

**SUMMARY**

The specific delivery of compounds to selected tissue and their activation by light *in situ* (thus sparing other uninvolved tissue) can dramatically increase the therapeutic index of a pharmacologic agent. Application of the techniques of molecular biology will permit the design of drugs with specific and selective activity. For example, in the
case of psoralens, the effects of substituents on photoadduct yields and the identification of those adducts responsible for mutagenic and possibly carcinogenic effects can lead to the rational design of more potent and safer psoralen derivatives.

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