A Comprehensive Photochemical and Photophysical Assay Exploring the Photoreactivity of Drugs**

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What are the interests of a pharmaceutical company in «Photochemistry of Drugs»? First of all drug-safety: safety by avoiding adverse effects in humans caused by exposure to light after the administration of certain drugs. Secondly, the photochemotherapy of skin diseases and human tumors is an active field of photomedicine. For this purpose pharmaceutical research should provide appropriate photosensitizers and the understanding of their mode of action. Furthermore, approaches to the protection of human skin against the harmful effects of solar radiation are of general interest and stimulate the development of novel sunscreens. The synthesis of sophisticated novel compounds using light as a highly selective reagent is an additional result of photochemical drug degradation studies in vitro. This is also relevant to the drug development process since toxic photodegradation products may contaminate the formulation, if the final drug or intermediary products are light-sensitive. This presentation, however, focuses on the photochemical and photophysical aspects of harmful effects to human skin, which may be produced by interaction of light and drug, and their possible prevention. Drug-induced photosensitization causing numerous adverse cutaneous responses or ocular complications in humans represents a severe side effect in the application of several pharmaceutically useful compounds. The different clinical manifestations observed in humans are classified as phototoxic, photoallergic, and drug-induced photosensitivity diseases. Typical photosensitizing drugs are selected from the literature, e.g. 8-methoxypsoralen, chlorpromazine, azathioprine, and nitroimidazoles (radiosensitizers), to demonstrate the diversity of the chemical reactions which are considered to be involved in the in vivo photosensitization reactions. – Consequently, a photochemical and photophysical in vitro assay is proposed, emphasizing the potential of photochemistry in rational drug design to minimize the aforementioned side effects. Connections to appropriate in vivo tests are considered. The screening of the antimalarial pyrimethamine combined with sulfadoxine and the antibacterial trimethoprim according to this assay led to a novel synthesis of 4-amino-s-triazinylketones via dye-sensitized photo-oxygenation of 2,4-diaminopyrimidines. Scope and limitations of this reaction will be discussed.

1. Introduction

«Photochemistry of drugs» represents an important field of interdisciplinary research involving photochemistry, photophysics, photomedicine, photobiology, and toxicology[1] (Scheme 1). During the past decade, interest in the reactions of human skin to light has been renewed as a consequence of the public's obsession with sun bathing or exposure to artificial light sources (Fig. 1), like solaria, television, day-light lamps, and home therapy units.

This change in human leisure habits may be designated as severe «light abuse». Additionally, the widespread use of certain drugs[2] led to a dramatic increase of drug-induced photosensitization diseases in humans. Representative structures initiating light-dependent skin diseases or eye damage are tetracycline antibiotics, sulfonamide diuretics like thiazides, polyhalogenated salicylanilides used as disinfectants, arylquinolines[2b] with outstanding antimalarial activity, and many others including cosmetics, detergents, dyes and food additives (Fig. 2). As can be deduced from this list of different chemicals, there exists no direct structure/activity relationship. A wide variety of chromophores may be involved in the photoreactions making a drug to an *in vivo* photosensitizer.

This is one of the reasons why we at Roche decided to design a photochemical and photophysical *in vitro* assay which enables us to specify the photoreactivity of drugs in view of their possible photosensitizing activity *in vivo*.

It is obvious that the evaluation of the photoreactivity of a chemical selected as a lead compound in pharmaceutical research has to be established at a very early phase of drug development to avoid future adverse light-induced effects.

2. Drug-Induced Photosensitization

2.1. Classification of drug-induced Photosensitization Effects

This leads directly to the question: what are the requirements for a drug to cause skin diseases or eye damage in connection with light?

Above all, the most trivial requirement is the absorption of light by the drug generating an electronically excited species which may cause biological damage (Scheme 2). This total process is called photosensitization. But there still remain some important limitations concerning the

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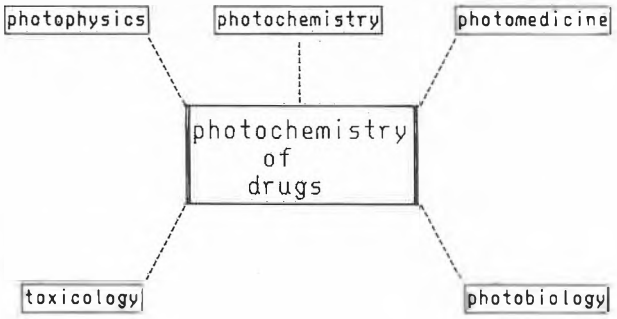
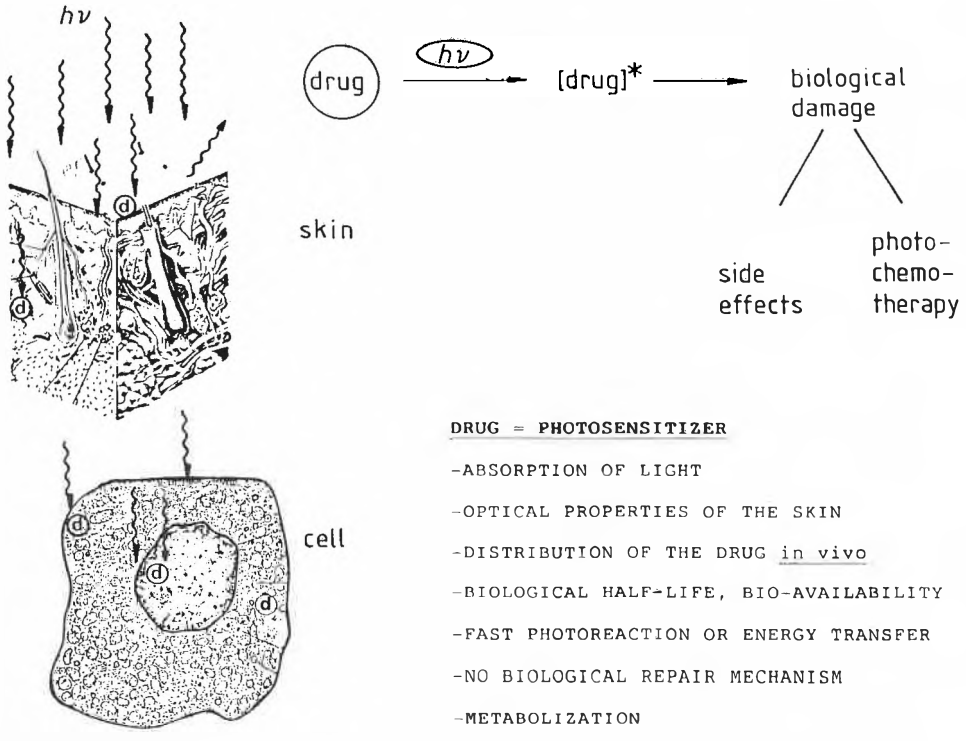


Scheme 1. Interdisciplinary research activities related to photochemistry of drugs.

Fig. 1. Artificial light sources.



Scheme 2. In vivo photosensitization.

optical properties of the skin, the distribution of the drug *in vivo* and its bioavailability. First of all, the light has to penetrate the skin to reach the cells which contain the drug, thus causing a substantial filter effect. Additionally, the drug should be distributed near enough to the surface of the skin or retina and to cell components that can be damaged by photoreactions, like the cell membranes or the DNA located in the nucleus. The biological half-life of the drug should be long enough to guarantee a critical concentration of the chemical and furthermore, the photoreaction or energy transfer must be sufficiently fast to compete with radiationless decay. Last not least, no efficient biological repair mechanism must exist. If all these requirements are met, the drug is called a photosensitizer in a medical sense.

The physiological and pathological consequences of the interaction of UV-light and a drug or an endogenous metabolite may include ocular damage, severe burning of the skin, painful sensation or erythema, edema, vesiculation, hyper- or hypopigmentation, and the development of severe skin cancer[1]. Besides these harmful adverse effects, photochemotherapy[1a, e] uses the controlled application of photosensitizers to treat certain skin diseases like psoriasis, herpes simplex, or different malignant tumors. PUVA-therapy applying psoralens in combination with UV-A irradiation (P + UV-A)[1f, g] and photodynamic therapy using hematoporphyrin derivatives and visible light[1h] are the most common applications of drug and light in chemotherapy for the treatment of psoriasis and cancer, respectively.

Further information about the optical properties of the human skin is presented in Fig. 3[1d].

It is found that short-wavelength UV-radiation is almost completely absorbed by the epidermis. However, the depth of light penetration increases with increasing wavelength. Therefore, visible light and infrared radiation reach the dermis and subcutis, respectively.

In order to classify the drug-induced photosensitization reactions in humans two broad categories have to be considered[1] (Scheme 3): the interaction of light with an exogenous or an endogenous agent.

Endogenous photosensitizers may be accumulated *in vivo* due to drug-induced disorders in metabolic or biosynthetic pathways. This may cause severe photosensitivity diseases, like drug-induced porphyrias which are characterized by an overproduction and accumulation of porphyrin intermediates of heme biosynthesis, e.g. protoporphyrin IX. Consequently, the photosensitization reaction observed in humans is due to the photodynamic action of the protoporphyrin IX and is probably mediated by reactive oxygen species.

On the other hand, light and exogenous agents like contact photosensitizers or systemically administered drugs may lead to

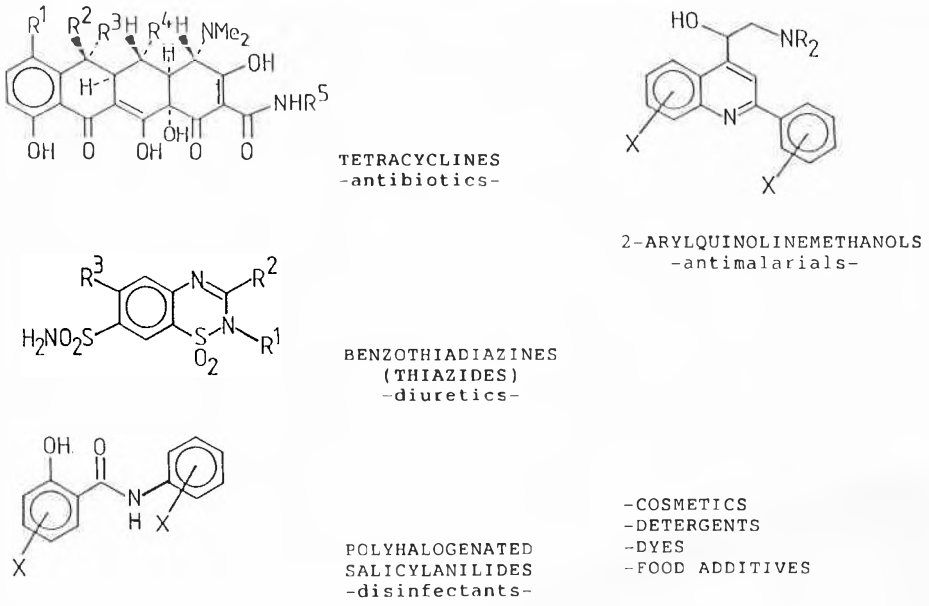


Fig. 2. Representative in vivo photosensitizers.

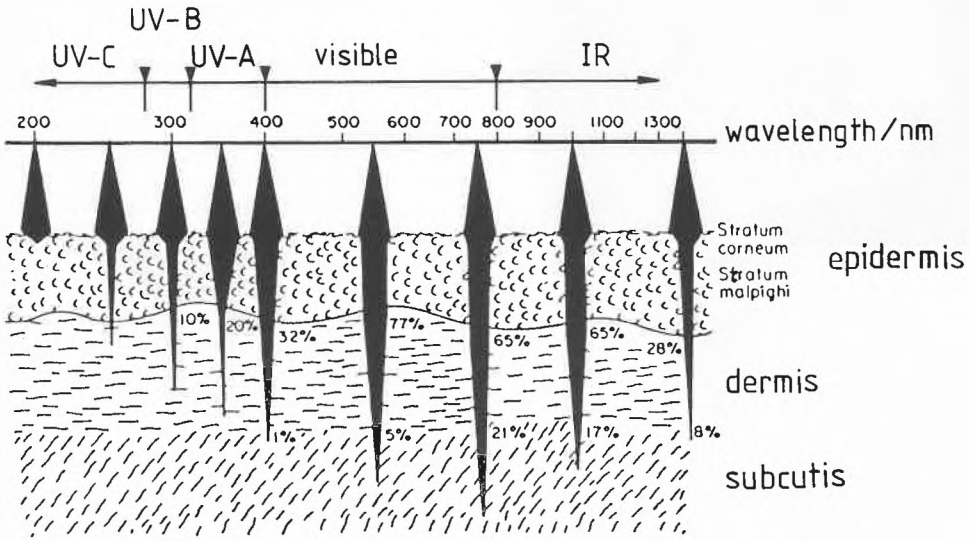


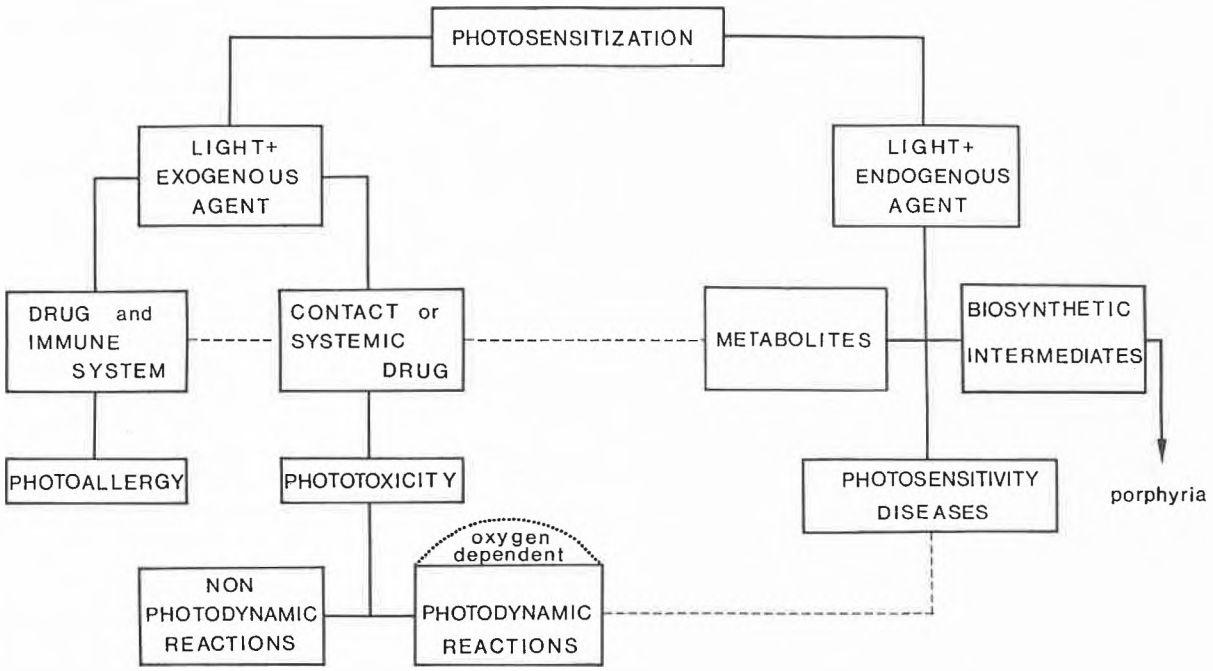
Fig. 3. Light skin penetration as a function of wavelength [1d].

two different types of photosensitization reactions in humans: to photoallergy involving the immune system leading to chronic disease or non-immunologic phototoxic responses. Phototoxic reactions themselves may be subdivided into non-photodynamic and photodynamic processes, the latter requiring oxygen for the production of cytotoxic reactive oxygen intermediates like singlet oxygen or the superoxide radical anion.

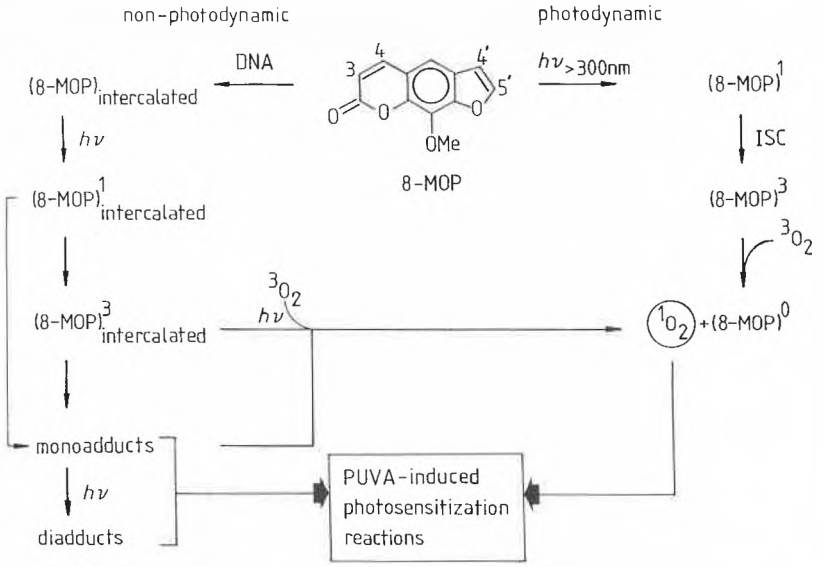
To demonstrate the diversity of chemical reactions which may be involved in photosensitization reactions in humans, four representative examples are selected from the current literature: 8-methoxypsoralen [3], chlorpromazine [4], azathioprine [5], and 5-nitroimidazoles [6].

2.2. Photochemistry of representative in vivo Photosensitizers

8-Methoxypsoralen [3] (Scheme 4) a furocoumarin and a typical phototoxic agent, is widely used as a dermatological drug in the clinical treatment of various skin diseases (cf. Section 2.1). The PUVA-photochemotherapy involves either topical or oral application of the drug followed by long-wavelength irradiation with UV-A ($\lambda = 400$–320 nm). Induction of edema and erythema as undesirable side effects are common. Under appropriate conditions, 8-methoxypsoralen intercalates into the DNA-double strands thus forming a non-covalent dark complex. Irradiation of this complex leads to the formation of cyclobutane-type mono- or diadducts involving the 5,6-double bond of DNA-pyrimidine bases and either the 3,4- or 4′,5′-sites of the furocoumarin [7]. This [2 + 2]-cycloaddition may occur from the singlet but more reasonably from the triplet state of the intercalated furocoumarin. The diadducts leading to so-called interstrand cross-links

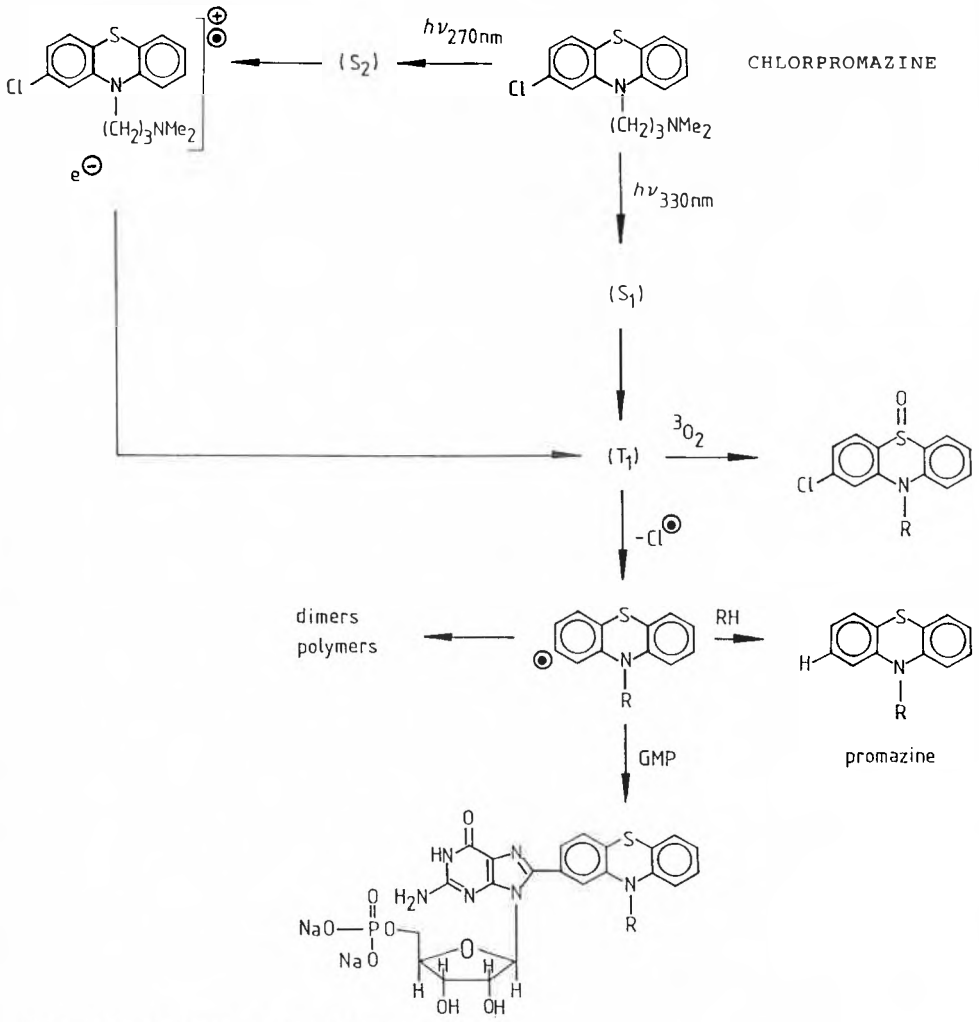


Scheme 3. Classification of drug-induced photosensitization reactions.



Scheme 4. Photoreactivity of 8-methoxypsoralen (8-MOP).

Generic and Systematic Names (CAS) of Drugs	
Azathioprine	6-[(1-Methyl-4-nitro-1*H*-imidazol-5-yl)thio]-1*H*-purine
Chlorpromazine	2-Chloro-*N*,*N*-dimethyl-10*H*-phenothiazine-10-propanamine
Fansidar® Roche	Sulfadoxine + Pyrimethamine
8-Methoxypsoralen	9-Methoxy-7*H*-furo[3,2-*g*]-1-benzopyran-7-one
Misonidazole	α-(Methoxymethyl)-2-nitro-1*H*-imidazole-1-ethanol
Pyrimethamine	5-(4-Chlorophenyl)-6-ethyl-2,4-pyrimidine-diamine
Sulfadoxine	4-Amino-*N*-(5,6-dimethoxy-4-pyrimidinyl)benzene-sulfonamide
Trimethoprim	5-[(3,4,5-Trimethoxyphenyl)methyl]-2,4-pyrimidine-diamine



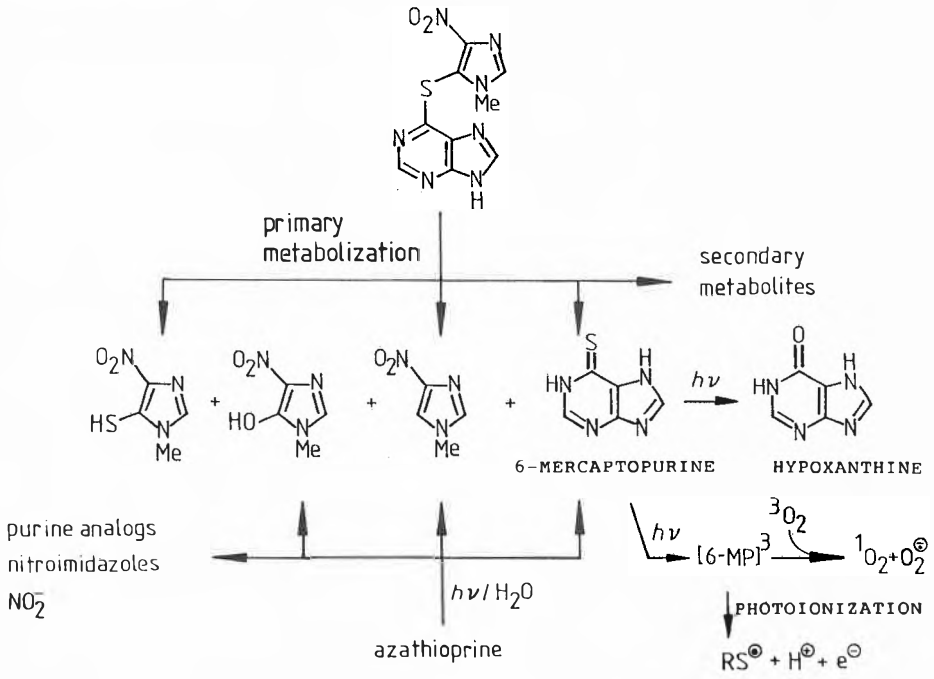
Scheme 5. Photoreactivity of chlorpromazine.

may be formed by absorption of a second photon by the furan-side monoadduct.

On the other hand, numerous investigators have found that psoralens additionally exhibit photodynamic activity[8] via photosensitized production of singlet oxygen. In the case of 8-methoxypsoralen the quantum yield of singlet oxygen production is relatively low. A value of $\Phi(^1O_2) = 0.0044$ in benzene[8c] was recently determined by *Truscott* and co-workers using time-resolved emission techniques for the detection of the characteristic luminescence of singlet oxygen at $\lambda = 1270$ nm applying acridine as a relative standard. Even intercalated triplet 8-methoxypsoralen and suitable model compounds for monoadducts have been found to generate singlet oxygen on irradiation under aerobic conditions. Both mechanisms, non-photodynamic and photodynamic, seem to be responsible for the PUVA-induced photosensitization reactions observed in humans, but further careful investigations are necessary.

Another clinical important drug is chlorpromazine[4] (Scheme 5), which often causes undesirable phototoxic and photoallergic side effects. Phenothiazine derivatives are used as tranquilizers, neuroleptics, and antihistaminica. The complex photochemistry of this compound can be explained by the formation of free radical intermediates which are generated via photodechlorination. Photo-ionization from the second excited singlet state does only occur at short-wavelength irradiation. Therefore, the involvement of the radical cation of chlorpromazine in photosensitization reactions *in vivo* is unlikely. The promazyl radical seems to be the precursor for dimers and polymers and for the dechlorinated parent promazine. This radical easily adds to guanosinemonophosphate (GMP) generating the corresponding coupling product, as was recently demonstrated by *Kochevar* et al.[9]. Consequently, chlorpromazine seems to be able to bind covalently to DNA upon irradiation. In conclusion, these results suggest that the promazine radical represents the active species causing cutaneous photosensitization[10]. This argument is supported by the fact that the parent promazine which cannot form radicals is an order of magnitude less phototoxic than chlorpromazine. Furthermore, trapping of triplet oxygen by triplet excited chlorpromazine leads to the formation of the corresponding sulfoxide, which is very interesting with regard to photoallergic effects[4b]. This process has been called «oxidative activation».

An even more interesting example of a photolabile drug giving rise to severe light-induced side effects including photo-carcinogenicity is azathioprine[5] (Scheme 6). This drug is most commonly used as an immunosuppressive agent for renal transplant recipients. The dermatological disorders induced by azathioprine and light originate from three different pathways: The most important way is the primary



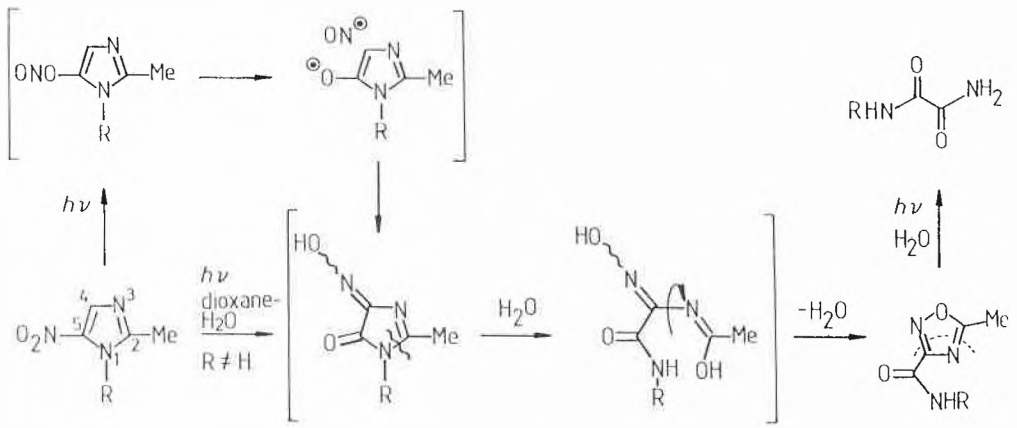
Scheme 6. Photoreactivity of azathioprine.

metabolization to compounds which are more photoreactive than azathioprine itself. The primary metabolites are the following 4-nitroimidazoles, methyl-nitrothioimidazole as well as methyl-nitroimidazole, and 6-mercaptopurine, respectively. Alternatively, *in vivo* damage may occur as a consequence of photo-decomposition of the drug leading to toxic or photoactive products. Photolysis of the drug in aqueous solution leads to the formation of hydroxy-methyl-nitroimidazole, methyl-nitroimidazole, and 6-mercaptopurine. Additionally, several other nitroimidazole derivatives and purine-analogs are formed besides the nitrite anion. From this scheme it is obvious that the metabolic and photodecomposition pathways to photoactive degradation compounds may be linked *in vivo*. A third way of action includes the photodynamic activity of 6-mercaptopurine. This compound was shown to produce singlet oxygen and the superoxide radical anion upon irradiation under aerobic conditions[5]. In deoxygenated solutions the formation of sulfanyl radicals could be detected. In addition, hypoxanthine is formed via secondary photolysis of 6-mercaptopurine. In conclusion, it has to be emphasized that the complex metabolic and photochemical degradation of azathioprine leads to a mixture of products exhibiting higher photoactivity than the parent drug. Furthermore, 6-mercaptopurine is an excellent electron donor, whereas nitroimidazoles are good electron acceptors. The complementary characteristics of these compounds may lead to electron transfer induced photosensitization reactions *in vivo*.

5-Nitroimidazoles (Scheme 7) represent a class of antimicrobial drugs[11] which have already been applied for over 25 years and their use is still increasing. Additionally, metronidazole and misonidazole are effectively used as γ-radiation sensitizers of hypoxic tumor cells in cancer radiotherapy[11]. The photoactivation of misonidazole was documented by the fact[6a, b] that its toxicity to various cellular systems is enhanced by irradiation with UV-A light, thus acting as a photosensitizer. Recently, *Pfoertner* et al.[6c] reported the formation of 1,2,4-oxadiazoles during the photolysis of 5-nitroimidazoles in aqueous medium. This interesting photo-rearrangement was studied in a series of widely used 5-nitroimidazole drugs, substituted at the N-1 position. The mechanism of this transformation involves the photo-initiated rearrangement to the corresponding nitrites. Cleavage of the N–O bond leads to the 4,5-dione-4-oximes which are subsequently hydrolyzed across the 1,2-C–N bond to an acyclic intermediate. Subsequent elimination of water leads to the 1,2,4-oxadiazoles in about 30 percent isolated yield. Secondary light-induced hydrolysis gives rise to the substituted oxalic acid diamides. The labile intermediate 4,5-dione-4-oximes could be isolated and identified spectroscopically in two cases.

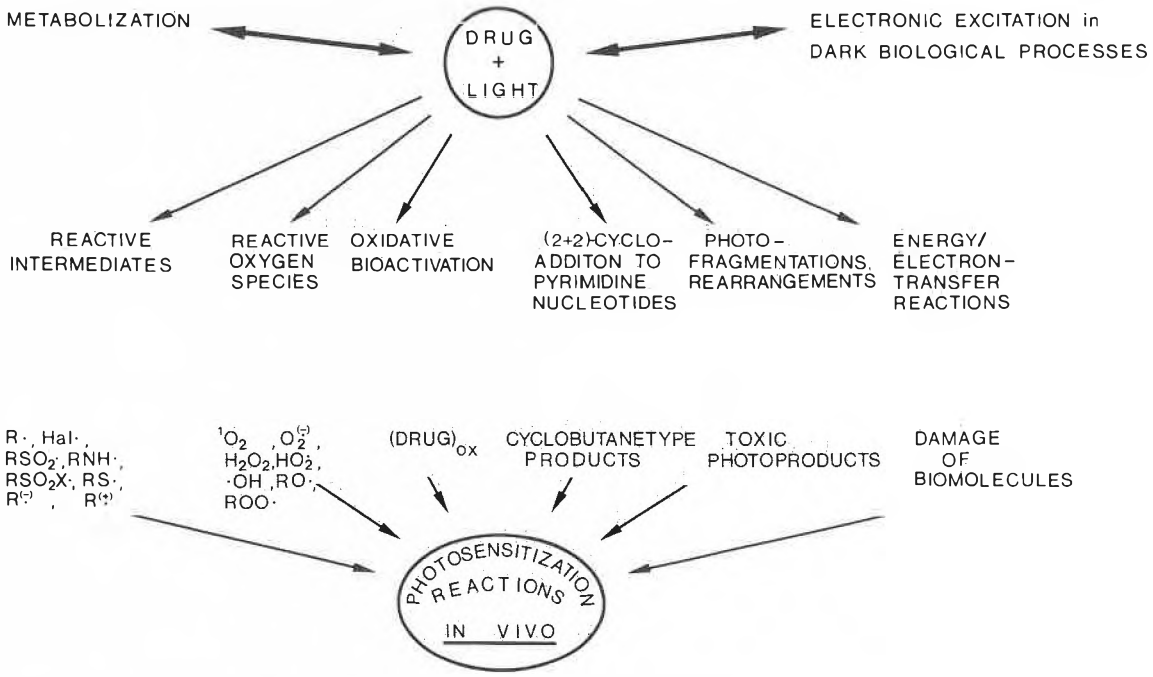
2.3. Photoreactions involved in Photosensitization

So far the diversity of the chemical and photochemical reactions which may cause photosensitization responses including



R = Me	Dimetridazole	isolated	(X-ray) 31%
CH_2CH_2OH	Metronidazole	–	30%
CH_3CHCH_2 OAc	–	–	31%
CH_2CHCH_3 OH	Secnidazole	–	31%
CH_3CHCH_2Cl OH	Ornidazole	isolated	32%

Scheme 7. Photoreactivity of 5-nitroimidazoles.



Scheme 8. Photoreactions causing photosensitization reactions in vivo.

photo-carcinogenic and photo-mutagenic reactions *in vivo* has been demonstrated. These reactions are summarized in Scheme 8 and represent the basis of our photochemical and photophysical assay.

Thus, the action of light on a drug may produce different free radical intermediates, radical anions, radical cations or cytotoxic reactive oxygen species like singlet oxygen or the superoxide radical anion or a series of substituted oxygen radicals[12]. As a consequence, photo-oxidation of proteins, cell membranes, unsaturated fatty acids, cholesterol, and nucleic acids may cause cell inactivation. Furthermore, light-induced oxidative bioactivation[13] may lead to an oxidized form of the drug which may induce phototoxic or photoallergic effects. As was demonstrated (cf. Section 2.2), psoralens cycloadd to the pyrimidine nucleotides leading to cyclobutane-type photoproducts which causes genotoxic effects and the inhibition of DNA replication. Additionally, photorearrangements and photo-fragmentations, as well as electron or energy transfer processes have to be considered to cause *in vivo* photosensitization reactions. This complex puzzle of interactive reactions might be complicated by metabolization of the drug to photoactive or toxic degradation products. In addition, the possible biochemical generation of electronically excited species in dark biological processes has to be considered[14].

3. Design of a Photochemical and Photophysical Assay

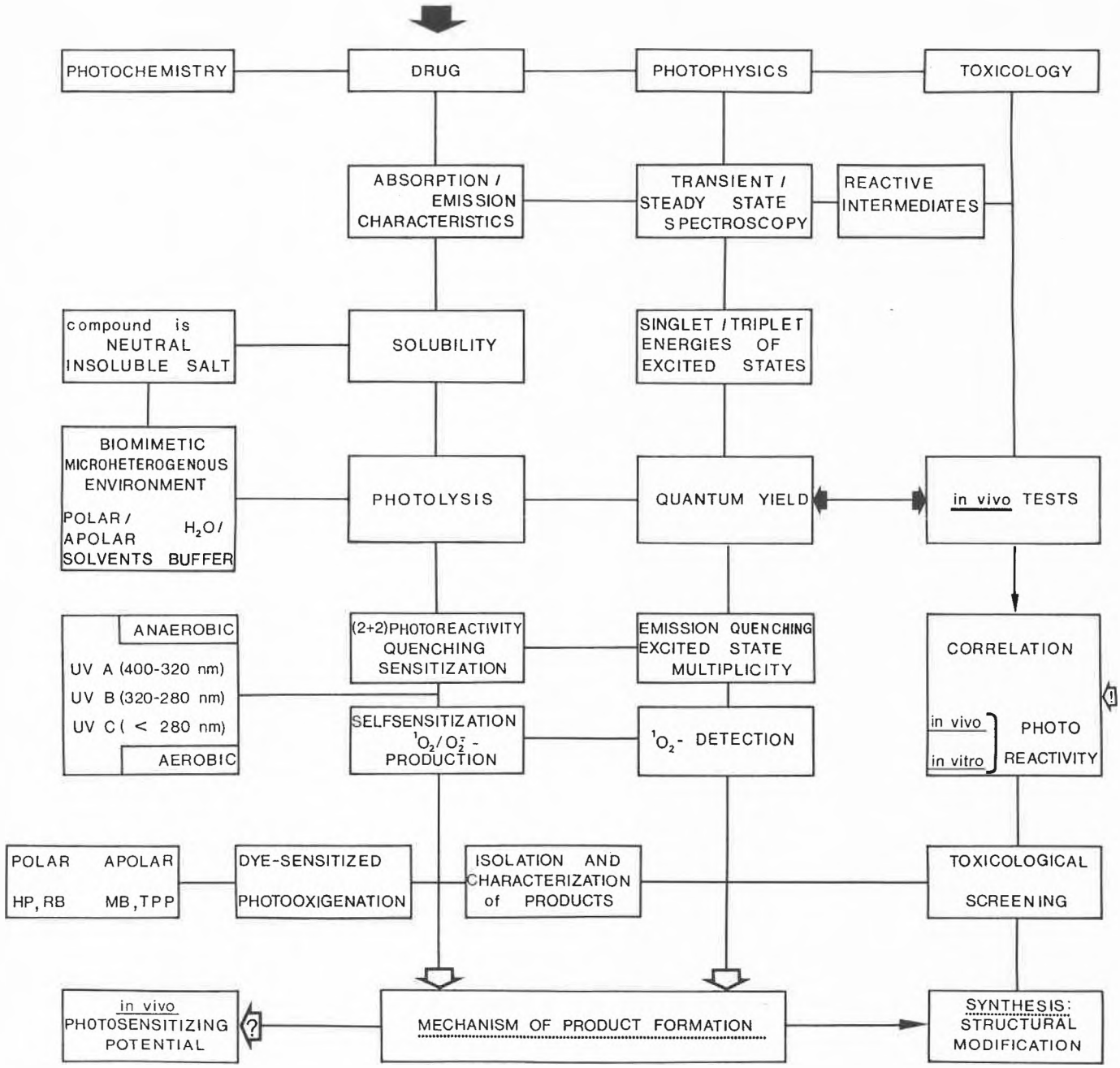
On this basis we developed the following photochemical and photophysical assay, which is discussed briefly (Scheme 9): Photochemical and photophysical methods should be combined to get a realistic description of the photochemistry of a certain drug. Absorption and emission characteristics in combination with transient or steady state spectroscopic methods are necessary to detect and specify possible reactive intermediates. Additionally, the singlet and triplet energies of the compound are of considerable interest to understand the mechanism of the photoreaction. In practice, the solubility of a drug may be a limiting factor influencing further investigations. Dependent on its solubility we try to «mimic» biological conditions using aqueous solutions or microheterogeneous systems[15] like micelles, liposomes or cyclodextrines. Photolysis in polar or apolar organic solvents leads to additional information. Then we have to check the photo-behaviour of the drug under aerobic and anaerobic conditions using physiological important wavelengths of irradiation like UV-A, UV-B or UV-C. Here it is very important to assess the [2 + 2]-photoreactivity of the drug in the presence of suitable biomolecules like thymidine, or to establish its self-sensitizing potential. Quenching and sensitization experiments in connection with emission quenching will help to characterize the multiplicity of the excited state which is involved in the photoreaction.

Furthermore, it is very important to establish the photodynamic potential of the substrate to generate excited oxygen species[16]. This may be achieved either chemically using specific quenchers for singlet oxygen, like diazabicyclo[2.2.2]octane (DABCO), superoxide dismutase[17] for the superoxide radical anion, or photophysically by detection of the characteristic luminescence of singlet oxygen[18]. Finally, we routinely investigate the behaviour of drugs against singlet oxygen applying photooxygenation conditions using hematoporphyrin or rose bengal as sensitizers in polar solvents and for example methylene blue or tetraphenylporphin in apolar media. After isolation and characterization of the photolysis products they have to be submitted to a broad toxicological screening to establish their toxic potential.

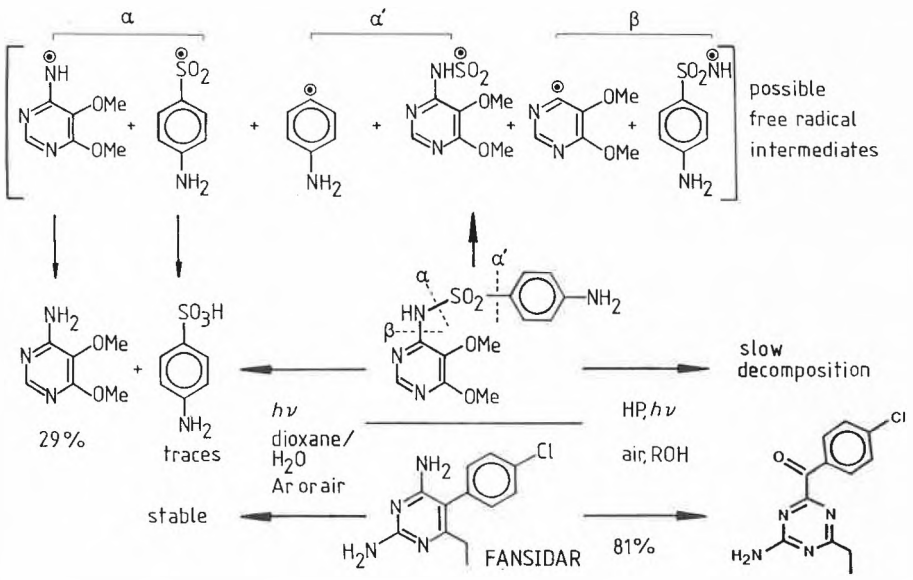
But the most important link between photochemistry, photophysics, and toxicology is the quantum yield of product formation or loss of starting material[2b, d] in relation to *in vivo* tests and appropriate biochemical screening tests [19,20]. This may result in a correlation between *in vivo* phototoxicity, characterized by the minimum erythema dose (MED) and the quantum yield of drug decomposition providing important structure/activity relationships.

The results of our assay lead to the establishment of a distinct mechanism of product formation. Thus, we should be able to estimate the *in vivo* photosensitizing potential of a certain drug from its *in vitro* photochemical behaviour[20]. Furthermore it has to be emphasized that the results of this assay may lead to a synthetic program which attempts to lower the photoreactivity of the compound by structural modification. These efforts should be integrated into rational drug design at a very early phase of drug development.

The criteria of subjecting a drug or a potential drug candidate to our *in vitro* photo-assay are the following: compounds which are developed for *long-term medication* and/or compounds which might be administered in *high dosage*, thus generating a *high blood concentration* during medical treatment. Finally, *environmental criteria*, i.e. medication in countries near the equator, must be considered.



Scheme 9. In vitro photo-assay.



Scheme 10. Photoreactivity of Fansidar® (= sulfadoxine + pyrimethamine).

4. Screening of Sulfadoxine and Pyrimethamine

In this section, the photoreactivity of the widely used antimalarial drug Fansidar® Roche (Fig. 4), based on our *in vitro* photoassay will be discussed. This drug consists of a mixture of two different pyrimidines, the sulfonamide sulfadoxine and pyrimethamine (the latter is a 2,4-diaminopyrimidine). The administration of Fansidar® may lead to severe light-induced adverse cutaneous effects in patients known to react allergic to sulfonamides [21].

The UV spectra of the two pyrimidines are quite similar, exhibiting two broad absorption bands. The long-wavelength absorption extends to about 320 nm as observed in the case of trimethoprim, an analogous 2,4-diaminopyrimidine. The emission characteristics of these compounds, e.g. fluorescence and phosphorescence, were used to determine the singlet and triplet energies of their lowest excited states.

As can be seen from the table (Fig. 4), the singlet energies of all three compounds are in the range of 91–95 kcal/mol. The triplet energies are probably lower than 49–52 kcal/mol.

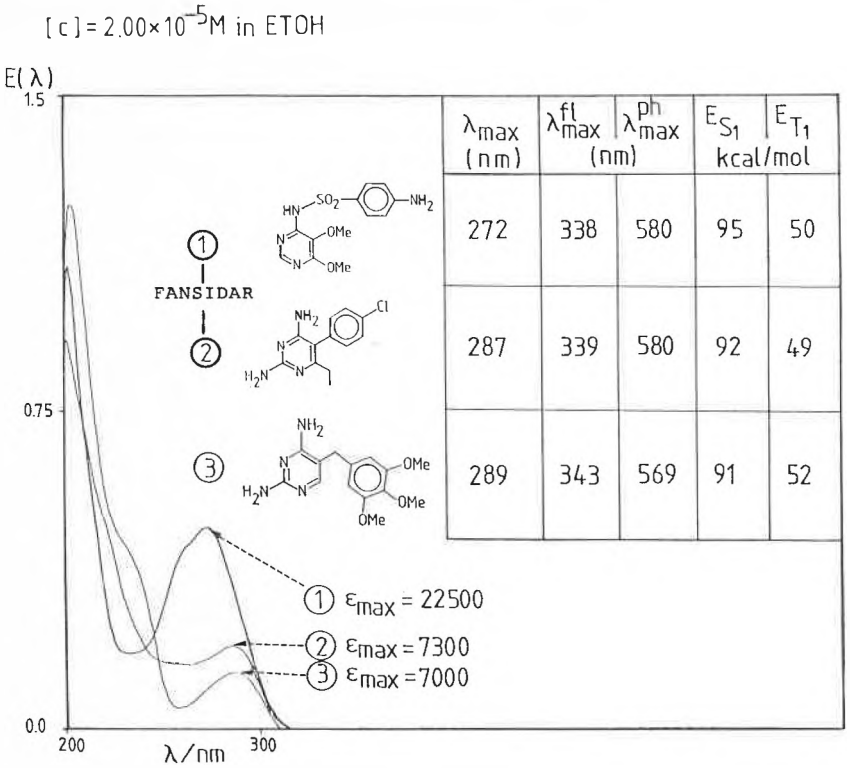
In spite of their photophysical similarities the photochemistry differs considerably (Scheme 10). Whereas pyrimethamine is stable towards photolysis in dioxane/water mixtures under argon or air, sulfadoxine shows rapid photodegradation to a complex mixture of products. Photoinitiated alpha-, alpha'- or beta-cleavage may lead to a variety of radical intermediates. These may dimerize, polymerize or directly react with cell components *in vivo*. We were able to isolate the products of S–N bond cleavage, 4-amino-5,6-dimethoxypyrimidine in 29% yield besides traces of sulfanilic acid. Thus, the photochemistry of sulfadoxine seems to be in accordance with the well-known photochemistry of sulfonamides[22]. On the other hand, sulfadoxine is stable towards photooxygenation using hematoporphyrin (HP) as sensitizer in ethanol or methanol.

Photo-oxygenation of pyrimethamine applying the same conditions led to the clean formation of a 4-amino-s-triazinyl-ketone in 81% chemical yield. Additionally, the mixture of sulfadoxine and pyrimethamine was photolyzed under analogous conditions leading slowly to the same distribution of products as determined for the individual components of Fansidar®. In order to understand the formation of the novel class of s-triazinyl-ketones we synthesized a variety of homologous pyrimidines containing different substituents at the 2-, 5-, and 6-position, respectively (Scheme 11).

Irradiation of the 2,4-diaminopyrimidines with hydrogen, methyl, phenyl at the 6-position and phenyl substituted at the 5-position of the pyrimidine ring, dissolved in ethanol or methanol in the presence of air and a sensitizer led to the formation of the corresponding 4-amino-s-triazinyl-ketones in good to excellent chemical yields. The original paper concerning this novel singlet oxygen reaction has been meanwhile published[23]. The reaction is effectively quenched using DABCO, confirming the involvement of singlet oxygen. In aprotic solvents like tetrahydrofuran or dichloromethane decomposition of the starting material takes place. Photo-oxygenation of the structurally modified pyrimidines with R^1 = adamantyl, R^2 = methyl and R^1 = ethoxycarbonyl, R^2 = hydrogen, the *N*-acetylated analog of pyrimethamine or the monoaminopyrimidine with R^3 = methyl, led to undefined decomposition of the 5-adamantyl compound; the three electron-deficient pyrimidines, however, did not appear to react with singlet oxygen.

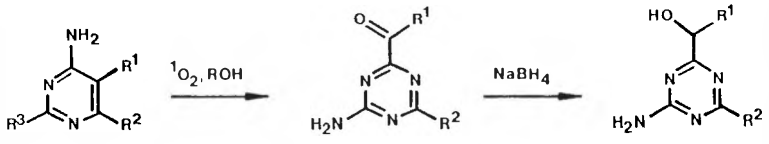
The structure of the s-triazinyl-ketones was unambiguously established by their reduction to the corresponding methanol derivatives using sodium tetrahydridoborat. The secondary alcohols could be isolated in 80% and 75% yield, respectively, as was exemplified by two reductions (Scheme 11). They reveal the characteristic ^{13}C-NMR and UV spectroscopic features of s-triazines.

The significant ^{13}C-NMR signals (Scheme 12) of the new 4-amino-s-triazinyl-ketones and the corresponding secondary alcohols are discussed using a schematic bar-graph. The assignment is convincingly based on two facts: firstly, the parent 6-unsubstituted compound exhibits a doublet at $\delta \approx 166$ for C-6 and secondly, the high-field singlet at $\delta = 165.8$ can be attributed to the amino-substituted C-4 in accord with literature data[24]. Furthermore, the C-4 resonances are invariable to substituent effects varying R^1 and R^2. The remaining singlets at 170 and 190 ppm have to be assigned to C-2 and the carbonyl resonance, respectively. The changes in the C-6 chemical shifts are typical for alkyl- or phenyl-substitution. A strong downfield shift for C-6 in the series of hydrogen, methyl, ethyl and a medium effect for phenyl is observed. The reduction of the



	λ_{max} (nm)	λ^{fl}_{max} (nm)	λ^{ph}_{max} (nm)	E_{S_1} kcal/mol	E_{T_1} kcal/mol
①	272	338	580	95	50
②	287	339	580	92	49
③	289	343	569	91	52

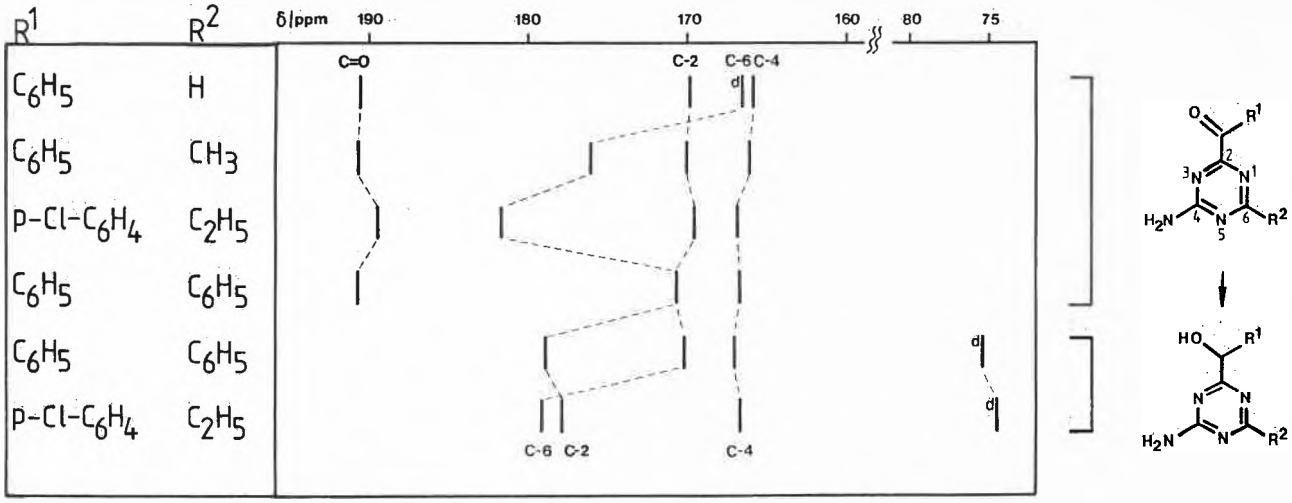
Fig. 4. Photophysical data of sulfadoxine, pyrimethamine, and trimethoprim.



R^1 = p-ClC$_6$H$_4$,	R^2 = C$_2$H$_5$	81%	80%
R^1 = C$_6$H$_5$,	R^2 = H	46%	–
R^1 = C$_6$H$_5$,	R^2 = CH$_3$	83%	–
R^1 = C$_6$H$_5$,	R^2 = C$_6$H$_5$	75%	75%
R^1 = adamantyl,	R^2 = CH$_3$	decomposition	
R^1 = CO$_2$C$_2$H$_5$,	R^2 = H		
R^1 = p-ClC$_6$H$_4$,	R^2 = C$_2$H$_5$, R^3 = NHCOCH$_3$	no reaction	
R^1 = C$_6$H$_5$,	R^2 = CH$_3$, R^3 = CH$_3$		

(R^3 = NH$_2$ for the first four rows)

Scheme 11. Photo-oxygenation of 2,4-diaminopyrimidines.



Scheme 12. Schematic bar-graph of the ^{13}C-chemical shifts of the new s-triazines.

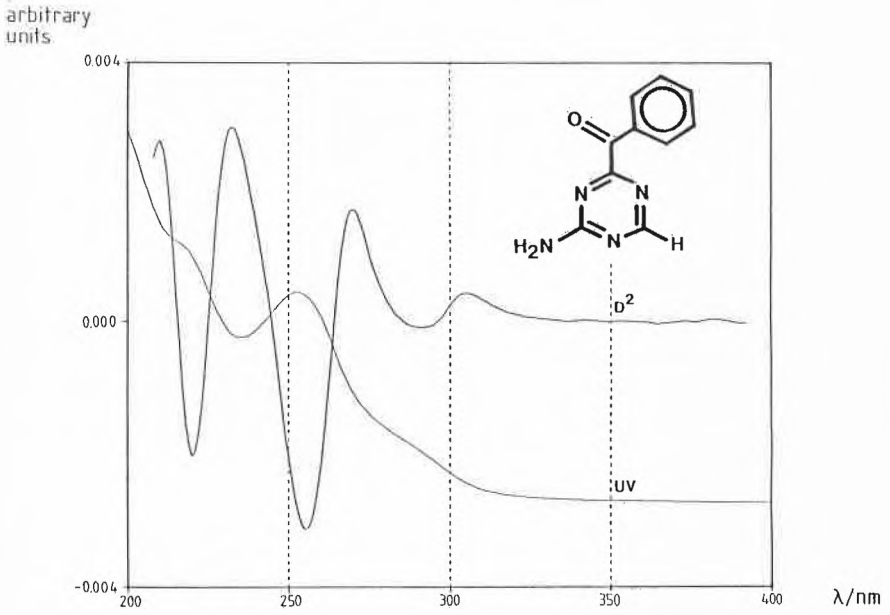


Fig. 5. UV- and corresponding second-derivative (D^2) spectra of 4-amino-s-triazin-2-yl phenyl ketone.

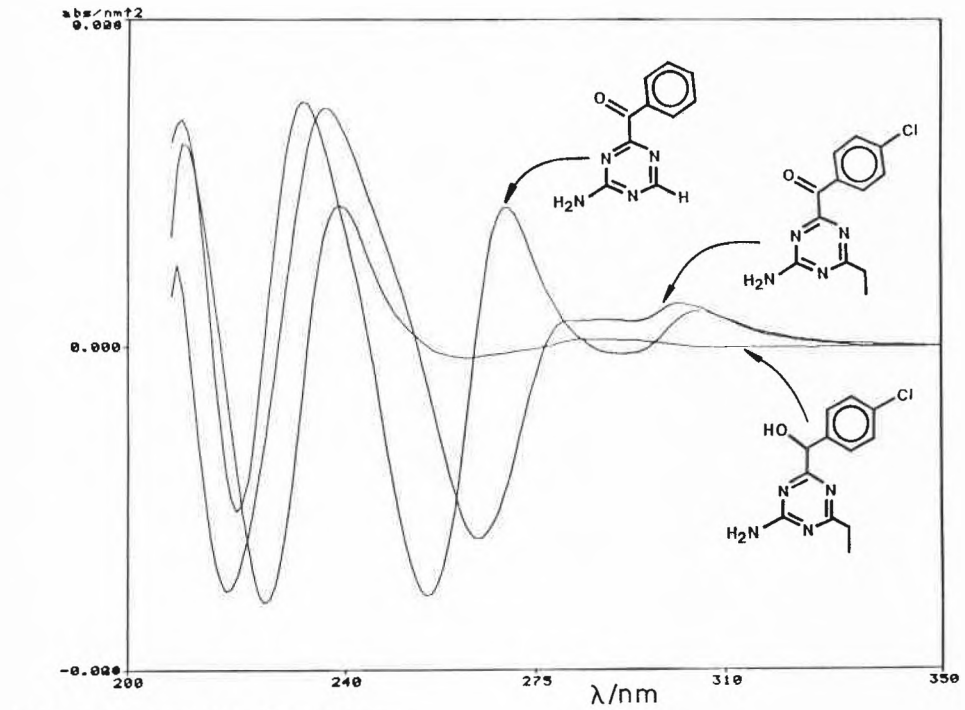
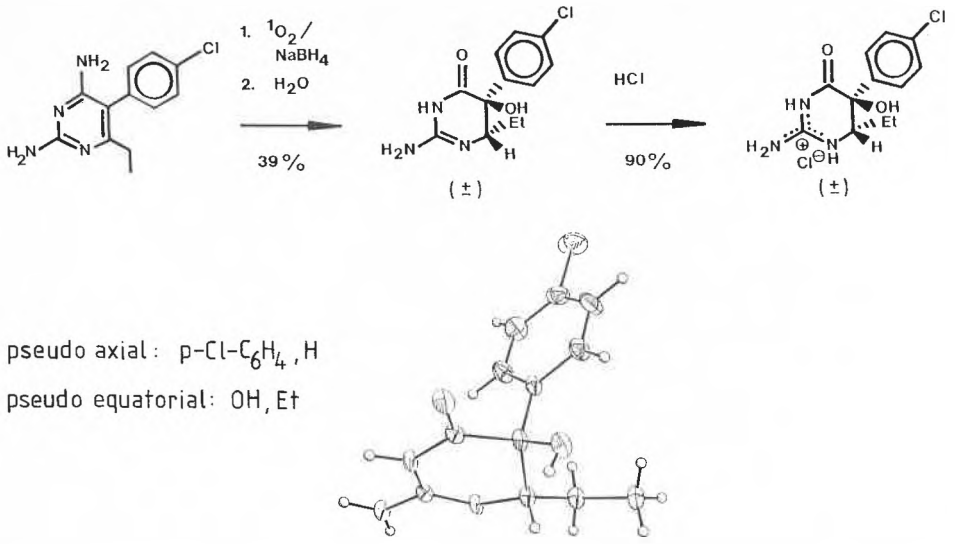


Fig. 6. D^2-UV spectra of s-triazines.

carbonyl group to the corresponding alcohols leads to a considerable downfield shift of the C-2 resonances and the appearance of the characteristic exocyclic secondary alcohol doublets. The negative inductive effect of the *p*-chloro substituent is evident causing a slight upfield shift of the exocyclic carbonyl resonances and the secondary alcohol doublets, as well as the C-2 signals of the triazinyl moiety.

An efficient method for the identification of s-triazines represents UV spectroscopy[25] (Fig. 5). For example, the UV spectrum of the 6-unsubstituted 4-amino-s-triazinyl-ketone consists of two bands located at 210–230 nm and 250–260 nm exhibiting a broad shoulder at 280–300 nm. The location of these absorption bands is relatively invariable to substitution effects. Still, the differences of the absorption maxima can be enhanced by the second-derivative technique[26]. The D^2-spectrum of the parent s-triazine exhibits two sharp minima representing the broad diffuse absorption maxima of the UV spectrum. All other s-triazines have similar D^2-spectra which therefore can be used as fingerprints for their structural identification. This is illustrated in Fig. 6 showing the D^2-spectra of the parent 6-unsubstituted s-triazine in comparison to the *p*-chlorophenyl compounds. The reduction of the carbonyl group to the secondary alcohol leads to a slight bathochromic shift of the short-wavelength absorption and the long-wavelength absorption disappears completely.

In order to get further information about the novel photo-oxygenation reaction of 2,4-diaminopyrimidines, we photooxygenated pyrimethamine under reductive conditions (Scheme 13) in the presence of six equivalents of $NaBH_4$ in ethanol at room temperature. The 5-hydroxy-4-dihydropyrimidinone was isolated from the complex reaction mixture after aqueous work-up in 39% chemical yield. The structure of this compound was unambiguously confirmed by X-ray analysis. It clearly reveals the pseudo-axial configuration for *p*-chlorophenyl and hydrogen and on the other hand the pseudo-equatorial configu-

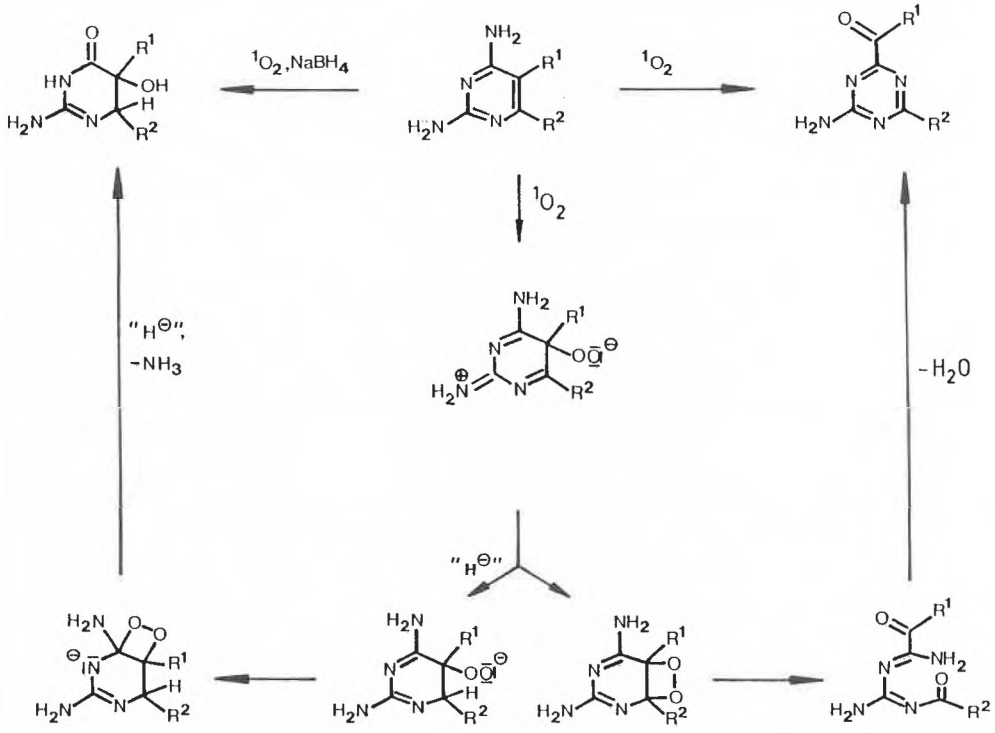


Scheme 13. Photo-oxygenation of pyrimethamin in the presence of sodium tetrahydridoborat.

ration of the hydroxy- and the ethyl-group, respectively. The free base of this interesting substituted dihydropyrimindinone could be smoothly transformed to the salt by dissolving in hot concentrated aqueous hydrochloric acid and collection of the colourless crystals which precipitated from the solution after cooling to room temperature.

Our experimental results concerning the dye-sensitized photo-oxygenation of 2,4-diaminopyrimidines suggest the following mechanistic scheme explaining the formation of the isolated products (Scheme 14): electrophilic attack of singlet oxygen at the 5-position of the 2,4-diaminopyrimidine gives a polar zwitterionic intermediate which is stabilized by the participation of the 2-amino group via its electron donating ability. Protic reaction conditions favour the formation of the unstable dioxetane intermediate which cleaves and rearranges to the final product. The facile reduction of the dipolar hydroperoxy intermediate by hydride attack at C-6 produces the anionic hydroperoxy species which should be able to form the unstable dioxetane intermediate substituted with an amino group at C-4. Subsequent reduction to the 4,5-diole and elimination of ammonia leads to the final product.

Dihydropyrimidines and s-triazines are widely used as pharmaceuticals[27] or agrochemicals[28], respectively.



Scheme 14. Proposed mechanism for the photo-oxygenation of 5-aryl-2,4-diaminopyrimidines.

5. Photo-Oxygenation of Trimethoprim

Our knowledge about the reactivity of 2,4-diaminopyrimidines against singlet oxygen was very helpful to understand the mechanism of the photo-oxygenation of the antibacterial trimethoprim which leads in 33% chemical yield to the formation of 3,4,5-trimethoxybenzaldehyd (Scheme 15). On the other hand, trimethoprim is stable towards photolysis under argon atmosphere and decomposes slowly in the presence of air. Isolation of additional products from the complex reaction mixture (Scheme 16) revealed the formation of s-triazines, but the mass balance was still poor, i.e. 55%. A reasonable mechanism which explains the formation of these products may involve the initial formation of a 4-amino-s-triazinyl-ketone in analogy to the 5-aryl substituted 2,4-diaminopyrimidines. Enolization and subsequent photo-oxygenation of the intermediate enole may lead to an unstable hydroxydioxetane. Cleavage of the dioxetane moiety according to path ⓐ explains the formation of the trimethoxybenzaldehyde and of the 4-amino-s-triazinylcarboxylic acid. This acid seems to be a reasonable precursor for 5-azacytosine which may be formed via decarboxylation and subsequent oxidation of the 2-amino-s-triazine. Esterification of the acid during silicagel chromatographic isolation using methanol as solvent led to the isolated methyl ester.

Nucleophilic solvent attack (EtOH) via path ⓑ leads to the incorporation of the ethoxy group and elimination of hydrogen peroxide. On the other hand, attack of ethanol via path ⓒ leads to the elimination of the amino-s-triazine and the formation of an α-hydroperoxy-ester leading to the corresponding trimethoxy-mandelate.

Up to now we discussed the reactivity of 2,4-diaminopyrimidines against electrophilic singlet oxygen. But the question arises, if these drugs are able to generate singlet oxygen in the presence of light and air? That means, do they exhibit photodynamic activity? These questions have to be answered in order to classify their photoreactivity *in vivo* as photodynamic or non-photodynamic. We examined this possibility (Fig. 7) by performing an intermolecular competition experiment using tetramethylethylene as an efficient singlet oxygen trap[16a]. The formation of hydroperoxydimethylbutene during aerobic irradiation of the drugs in the presence of tetramethylethylene was monitored using quantitative gaschromatography with 1-decanol as internal standard. Trimethoprim, sulfadoxine, and pyrimethamine led after 23 h to almost the same concentration of the hydroperoxide. Its formation was efficiently quenched in the presence of 0.5 equivalents of DABCO indicating the involvement of singlet oxygen. As a reference system we used the rose bengal sensitized photo-oxygenation of tetramethylethylene which proceeded very fast. A concentra-

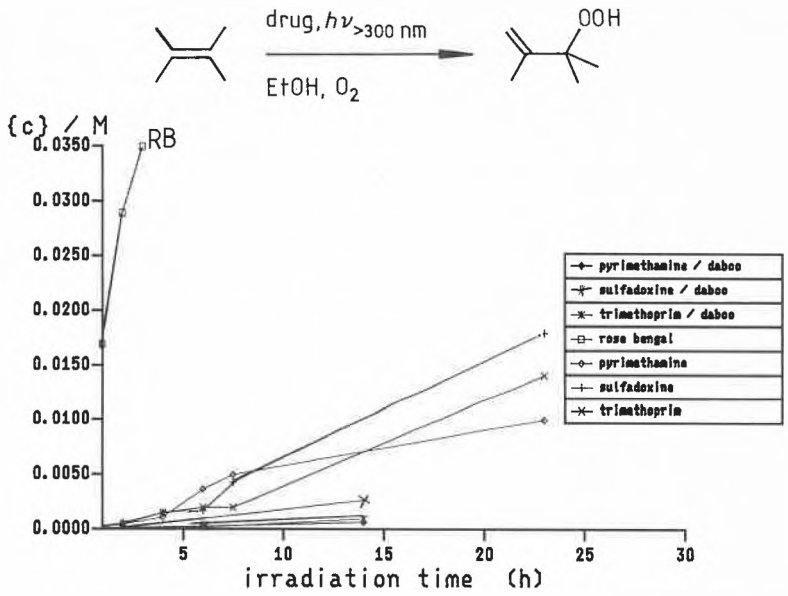
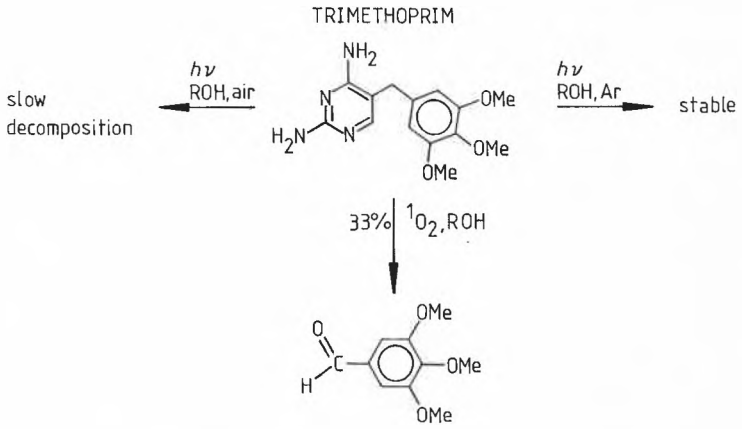


Fig. 7. Photodynamic activity of pyrimethamine, sulfadoxine, and trimethoprim.



Scheme 15. Photoreactivity of trimethoprim.

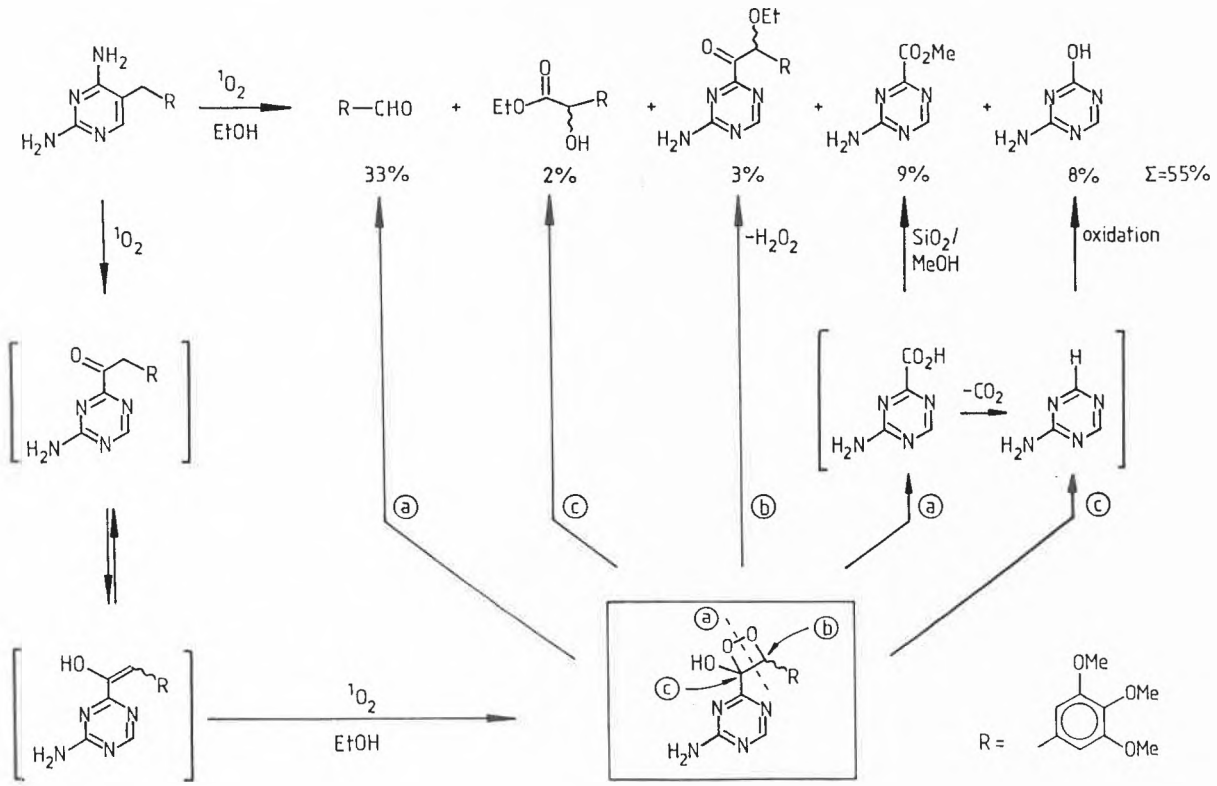
tion of the hydroperoxide which was almost three times higher as in the case of the drugs was already reached after 3 h of photo-oxygenation. This result clearly demonstrated that the production of singlet oxygen under aerobic conditions by 2,4-diaminopyrimidines is negligible. This is in accordance with the high triplet energies of about 50 kcal/mol of these compounds.

6. Conclusions and Outlook

In conclusion, it is obvious that the phototoxicity of Fansidar® is related to its sulfonamide component sulfadoxine and that the photodynamic activity of Fansidar® and trimethoprim is negligible. Trimethoprim itself exhibits no defined photochemistry and decomposes upon dye-sensitized photo-oxygenation under the formation of a complex mixture of s-triazines and 3,4,5-trimethoxybenzaldehyde as the main product. Additionally, we discovered a synthesis of novel 4-amino-s-triazinylketones by dye-sensitized photo-oxygenation of 2,4-diaminopyrimidines in protic solvents.

Furthermore, our *in vitro* «photo assay» leads to the complete description of the photoreactivity of drugs and enables us to estimate the possible *in vivo* photosensitizing potential of these compounds.

The present article demonstrates the enormous potential of our *in vitro* photoassay to establish the mechanisms of drug-induced photosensitization in humans. This methodology should be applied at a very early phase of drug development to



Scheme 16. Photo-oxygenation of trimethoprim.

avoid undesirable light-induced side effects. Future activities should concentrate on the intensification of interdisciplinary cooperation with biochemists, physicians, and toxicologists to obtain detailed information about the biochemical and toxicological mechanisms of action of photosensitization in humans. Microbiological screening tests[19c,d] and biochemical methods[20] including protein binding studies applying fluorescence techniques[29] are an important extension of our *in vitro* photo-assay in order to minimize *in vivo* tests with animals.

And now, we should be very optimistic that interdisciplinary research involving photochemistry, photophysics, photomedicine, photobiology, and toxicology is able to solve the problems related to drug-induced photosensitization in humans and therefore the author agrees with the saying: «Where light exists, there is hope!»

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