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Photosensitization by selected anticancer agents

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Novel anticancer anthrapyrazoles and anthracenediones are available as alternatives to the cardiotoxic clinical agents, doxorubicin and daunorubicin. Certain representatives of these new classes of compounds possess photosensitizing properties. The structural features influencing the photophysical parameters of these agents are discussed. Photosensitizing reactions involving singlet oxygen production, free radical formation, decomposition of hydrogen peroxide and organic hydroperoxides, oxidation of certain biochemical electron donors, DNA damage and killing of human leukemic cells in vitro in the presence of photoactive anthrapyrazoles, anthracenediones and anthracyclines are described.

1. Introduction

Interest in photosensitization phenomena has increased enormously within the last two decades. One of the reasons for the particular attention paid to those processes is their biomedical significance. On the one hand, there is a long list of compounds, of both natural and synthetic origin, which are known to cause phototoxic and photoallergic reactions in animals including humans [1–4]. Among the compounds with these properties are food additives, herbicides, cosmetics, and certain types of drugs [4–8]. On the other, the interest is motivated by new biomedical applications of photosensitizing compounds in such areas as diagnosis and photodynamic therapy (PDT) of cancer and other abnormalities [9,10]. In this respect, PDT, utilizing hematoporphyrin derivatives (HPDs), as the photosensitizing agent, has played a pioneering role. Historically, however, the leading position in the medical application of photosensitizing compounds belongs to furocoumarins [11]. Although the primary utility of this approach initially seemed to be limited to readily accessible lesions, the development of new technologies, such as fiber optics and extracorporeal procedures, made it possible to extend the application of PDT to internal organs as well [12–14].

Progress in the development of instrumental techniques permitted insight into the early stages of photosensitization mechanisms, via energy transfer, including direct detection of singlet oxygen luminescence, and electron-transfer processes [10]. Interest in PDT stimulated the search for new photosensitizing agents with properties superior to those shown by HPDs. A potentially useful therapeutic agent should possess such features as low dark toxicity, selective accumulation in cancer cells, appropriate retention time, high absorptivity in the red portion of the spectrum, high yield of triplet state formation and long triplet lifetime. Numerous other factors, affecting the extent of the photodamage, originate from the effects of the specific, highly heterogeneous, in-

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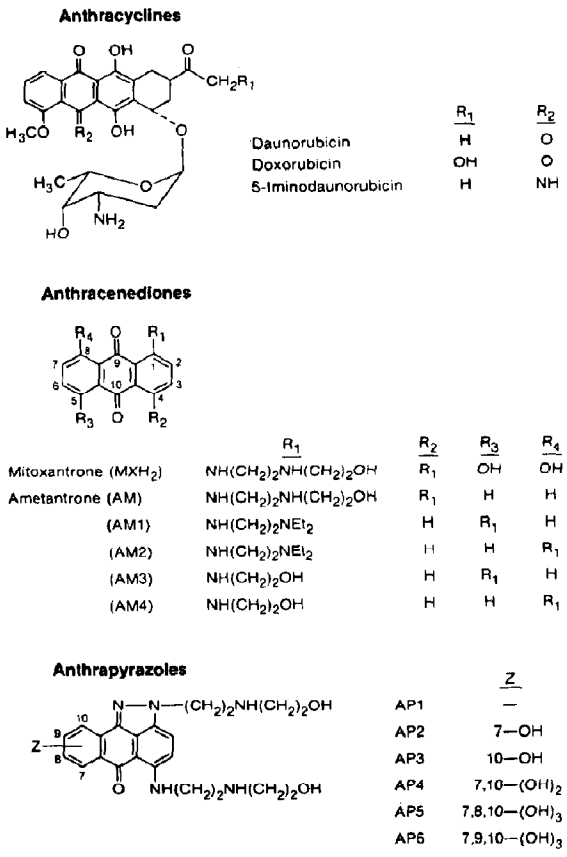


Fig. 1. Structures of anthracycline antibiotics, and anthracenedione and anthrapyrazole anticancer agents.

tracellular environment, modulating the photosensitizing properties of an agent. Because excellent review articles devoted to the latter topics are available (e.g., see refs. 15–17) they will not be discussed here. Instead, in this review we will summarize the photochemistry and photobiology of certain new classes of anticancer agents studied in this laboratory during recent years, and which show interesting photosensitizing properties. Although some of these new drugs are phototoxic to cancer cells in vitro their potential in PDT in vivo has yet to be demonstrated.

The new anticancer agents in question were developed as alternatives to the anthracycline drugs, whose photosensitizing capabilities are also summarized in this review. The anthracycline antibiotics, daunorubicin and doxorubicin (fig. 1), are among the most widely prescribed clinical anticancer agents. They suffer, however, an important limitation due to the risk of severe dose-related cardiotoxicity [18,19]. This deleterious side effect has been linked to the generation of oxygen-derived radicals via bioreduction of the quinone moiety of the drugs and subsequent redox cycling [20,21]. Therefore, the rationale in synthesizing chromophore-modified anthracycline analogues was to render the electron-deficient quinone in those chromophores more resistant to enzymatic reduction, while retaining structural characteristics of the chromophore necessary for DNA binding [22]. Anthrapyrazole (APs) and anthracenedione anticancer agents (fig. 1) are among the results of such efforts [23,24].

2. Anthrapyrazoles

The modification of the anthracycline chromophore to obtain the anthrapyrazole structure rendered certain examples powerful photosensitizers. Ethanolic solutions of APs show absorption maxima in the visible region between 470 and 516 nm, depending on the number and position of the OH substituents [26]. Some APs are capable of participating in type II reactions. Generation of singlet oxygen upon illumination of APs in ethanolic solutions was demonstrated using 1,5-dimethylfuran (DMFu) and NaN_3 as a singlet oxygen trap and as a quencher, respectively [25].

Marked differences among the APs in the ability to generate $^1O_2$ were related to the degree of hydroxylation of the chromophore. The presence of OH groups in the chromophore, in positions facilitating formation of intramolecular hydrogen bonds between -O-H...N = and/or -O-H...O = groups, apparently reduces the photosensitizing capabilities of APs. By analogy with many other cases, it may be suggested that the observed loss of the photoactivity is related to the presence of efficient mechanisms of nonradiative deactivation of excited state(s) in hydroxylated APs, shortening the lifetime and/or yield of the formation of triplet states. As an example, fig. 2 shows the rates of photosensitized oxygen consumption (via reac-

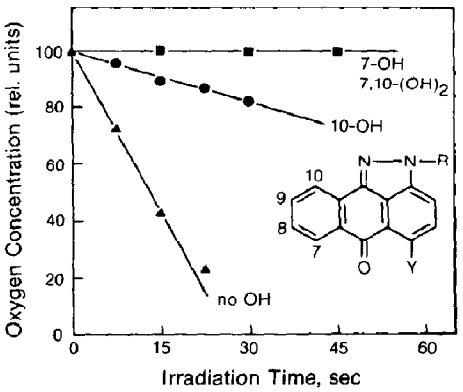
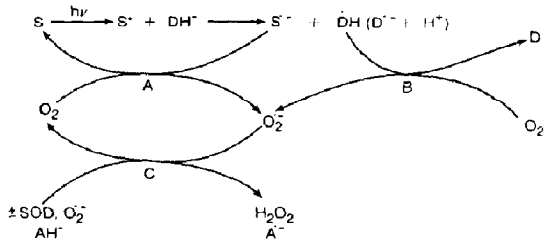


Fig. 2. Oxygen consumption photosensitized by different anthrapyrazole agents in ethanolic solutions in the presence of DMFu as a singlet oxygen trap: effect of hydroxyl substituents at positions 7 and 10 in the chromophore. Samples containing AP (approx. 0.55 mM), DMFu (1.5 mM) and TEMPO (0.2 mM) in 84% ethanol were illuminated at $\lambda \approx 480$ nm (approx. 10 W/m^2).

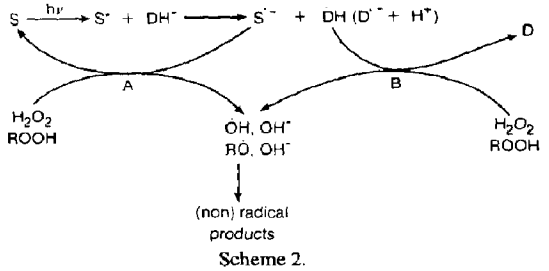
tion between $^1O_2$ and DMFu) for double-, mono- and nonhydroxylated APs. Direct proof of the generation of singlet oxygen by AP1 was obtained by detection of infrared luminescence (at 1270 nm) during illumination of this compound with blue light in ethanolic solution (R.D. Hall, NIEHS, unpublished data). No such emission was detected from the 7,10-dihydroxy-substituted agent (AP3).

Recent studies of the photosensitizing properties of APs have revealed a contribution from mechanisms other than $^1O_2$ generation. These processes are of type I and are observed during AP1-photosensitized oxidation of certain electron donors in aqueous solutions [26,27]. Oxidation of ascorbic acid, AH^-, during illumination of AP1 (approx. 480 nm) was accompanied by rapid formation of the ascorbyl radical, $A^{\cdot-}$, as established employing EPR techniques [26]. Concomitant production of hydrogen peroxide was demonstrated by catalase-sensitive oxygen consumption [26]. It was suggested that the reaction proceeds via electron transfer from AH^- to the triplet excited state of AP1 with subsequent reduction of oxygen, as shown in scheme 1 (S, photosensitizing agent; DH^-, sacrificial electron donor). A semiquinone radical from the AP1 molecule should also be produced although it has not been detected by EPR [26]. Indirect evidence for the formation of the anthrapyrazole-derived radical was obtained from EPR studies of the drug-photosensitized decomposition of inorganic and organic hydroperoxides (see below). However, a radical derived from an analogous compound, AP2 (fig. 1) was detected in pulse radiolysis experiments [28]. In the absence of oxygen this radical decayed by disproportionation with a second-order rate constant of 1.2×10^9 M^{-1} s^{-1} [28]. In aerated solutions the latter species reacted with oxygen, by electron transfer, with a rate constant of 1.8×10^9 M^{-1} s^{-1} [28]. The one-electron reduction potential of AP2 in the ground state was calculated to be $E_7^1 = -538 + 10$ mV [28]. It is anticipated that the one-electron redox potential for the photochemically most reactive compounds (such as AP1), will be equal to, or less negative than, that for AP2. Polarographic redox potentials for several APs in the ground state are listed in ref. 24.

Besides AH^-, other biochemical electron donors, such as DOPA and NAD(P)H were also shown to undergo photooxidation sensitized by AP1 [26,27]. In the latter case, formation of superoxide radical was detected using EPR and spin-trapping methods [27]. Illumination of AP1 with either of these substrates in aerated solutions produced hydrogen peroxide [26,27]. Hydroxyl radicals were readily produced upon illumination of AP1 in the presence of catalytic concentrations of Fe(III)/EDTA complex and one of the above reducing agents. Formation of free hydroxyl radicals was corroborated using ethanol or sodium formate as radical scavengers. In the presence of such additives DMPO adducts of either alcohol- or formate-derived radicals were observed. Production of OH radicals is maintained by the



Scheme 1.



Scheme 2.

light-stimulated formation of the Fenton system. The latter is accomplished in two steps: (i) reduction of O_2 to O_2^- (scheme 1, routes A and/or B), with subsequent dismutation to H_2O_2 and O_2 (scheme 1, route C), and (ii) light-enhanced electron transfer from an electron donor via semireduced drug or semioxidized substrate to the ferric complex. Therefore, the accelerated production of the OH species is possible when oxygen is depleted, otherwise it might prevent the reaction of the latter step.

An alternative mechanism for the formation of hydroxyl radicals by the photochemical decomposition of hydrogen peroxide was uncovered. This mechanism is not catalyzed by metal ions, but involves a direct electron transfer from the photoreduced sensitizer, AP^-, to the H_2O_2 molecule (scheme 2, route A). Illumination of deaerated solutions containing API, ascorbic acid and H_2O_2 produced the EPR spectrum of the ascorbyl radical, A^- [26]. The radical does not react rapidly with this peroxide and, as a result, its EPR amplitude increased when the concentration of H_2O_2 increased to 5 mM and then leveled off (fig. 3) (unpublished data). EPR spin-trapping measurements with DMPO showed that the formation of the A^- radical is concomitant with the production of the DMPO-OH adducts [26,27]. Again in the presence of ethanol or sodium formate, the EPR spectrum of the DMPO-OH adduct was replaced by spectra from hydroxyethyl- or formyl-DMPO adducts, respectively, confirming the generation of free hydroxyl radicals [26,27].

Organic hydroperoxides can be decomposed in similar photochemical reactions. For example, illumination of API, AH^- and *t*-butyl hydroperoxide in deaerated solutions generated the ascorbyl radical and, when DMPO was present, the DMPO-CH_3 adduct was detected (unpublished data). Scheme 2 shows possible reaction pathways for such a case. An alkoxy radical $RO^\cdot$ (in this case $(CH_3)_3O^\cdot$) formed via route A or B, undergoes subsequent spontaneous decomposition to acetone and the methyl radical [29] which is scavenged by the spin trap DMPO. These results demonstrate that both inorganic and organic peroxides can participate in photochemical redox reactions as electron acceptors and perhaps can replace oxygen in many light-driven electron-transfer processes. A similar mechanism of decomposition of hydrogen peroxide, *t*-butyl hydroperoxide and cumyl hydroperoxide by another radical, enzymatically formed daunorubicin semiquinone, has been described by Kalyanaraman et al. [30]. Such a reductive decomposition of peroxides was also observed in pulse radiolysis experiments [31,32] and is a major pathway in their metabolic transformations [33,34].

Single-strand breaks (ssb) can be introduced into DNA upon illumination of non hydroxylated, but not hydroxylated, APs [35]. The damage is enhanced by the presence of electron donors such as NADH, ascorbic acid or Fe(III)/EDTA complex and an oxygen-dependent mechanism of strand-break production was demonstrated. Under anoxic conditions, however, a second mecha-

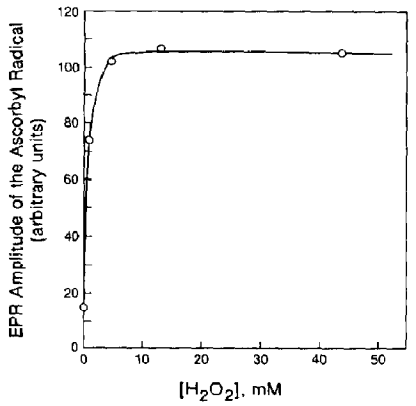


Fig. 3. Production of ascorbyl radicals during API-photosensitized decomposition of hydrogen peroxide. Sample contained API (0.33 mM), AH^- (1 mM) and H_2O_2 in deaerated phosphate buffer (pH 7.1). Illumination at $\lambda \approx 480$ nm.

nism involving drug and NADH molecules is also indicated [35]. Smooth sequence neutral photosensitized cleavage of DNA is observed analogous to hydroxyl radical 'footprinting' [36]. Anthrapyrazoles have been tested for their ability to photosensitize neoplastic cells in culture. Nonhydroxylated agent, AP1, induced photodamage to murine leukemic cells as determined by thymidine incorporation and cell viability studies [37].

3. Anthracenediones

Anthracenedione anticancer agents are another example of the efforts to synthesize less cardiotoxic chromophore-modified anthracycline analogues [38–40]. Mitoxantrone (MX) and ametantrone (AM) are the best known representatives of this class of compounds, and mitoxantrone is a clinically useful agent [40]. The structural formulae of MX and AM, and some structural analogues (AM1–AM4) are shown in fig. 1. Aqueous solutions of MX are intensely blue in color, and their absorption maxima, in the visible part of the spectrum, are at 610 and 664 nm. For AM the positions of the λ_{max} are at 548 and 626 nm. Neither MX nor AM shows photochemical reactivity when illuminated with visible light at their λ_{max} [41,42]. Their structural variants, however, viz., the 1,5- and 1,8-diamino-substituted anthraquinones, such as AM1–AM4 (fig. 1), were shown to be strongly photochemically active [42]. EPR spectra of radicals derived from AM1 and AM2 were recorded upon illumination of these agents in deaerated aqueous solutions in the presence of NAD(P)H (fig. 4a,b) [42]. More intense and better resolved EPR spectra were obtained from AM3 and AM4 analogs in DMF/aqueous mixture (fig. 4c,d), a result of the diminished aggregation of solutes in the presence of an organic solvent in the system [42].

Illumination of the photoactive anthracenediones and NAD(P)H caused changes in their absorption spectra. This is illustrated in fig. 5 for AM1. The decrease in the original absorbance at 518 nm is accompanied by the appearance of a new absorption band at 418 nm, ascribed to a hydroquinone form of the agent [42].

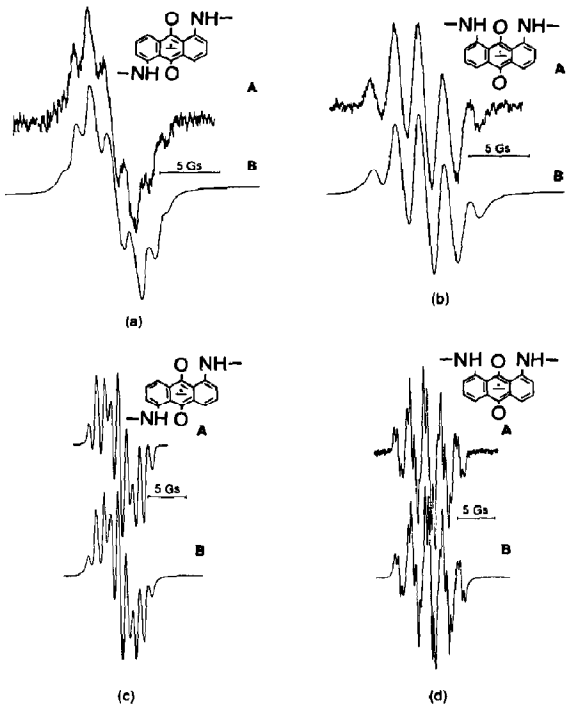


Fig. 4. EPR spectra of semiquinone radical anions from (a) AM1, (b) AM2, (c) AM3, and (d) AM4. Samples contained an appropriate aminoanthraquinone (0.64 mM), and NADH (0.4 mM) in deaerated buffer, pH 7.1 (a,b) or DMF/buffer (7:3, v/v) mixture (c,d) and were illuminated at $\lambda > 475$ nm. (A) Experimental spectra. (B) Simulated spectra using the parameters: (a) $a_H = 1.2$ G (4H), $a_H = 1.45$ G (2H); (b) $a_H = 2.1$ G (4H); (c) $a_H = 1.1$ G (4H), $a_H = 1.85$ G (2H); (d) $a_H = 2.4$ G (2H), $a_H = 1.8$ G (2H), $a_H = 0.45$ G (2H). From ref. 42.

In aerated solutions, superoxide radical, identified as a superoxide dismutase (SOD)-sensitive DMPO-O_2^- adduct formation, was readily generated [42]. The photosensitized oxidation of NAD(P)H and formation of O_2^- and H_2O_2 species occurs as depicted in scheme 1. The same system produced hydroxyl radicals in the presence of catalytic concentrations of the Fe(III)/EDTA complex [42]. Involvement of the hydrogen peroxide in this process was confirmed by a catalase assay.

Single-strand break formation in DNA in the presence of AM1 and AM2 and NADH or ascorbic acid upon activation with light was reported [43]. While the presence of an oxidizable cofactor, such

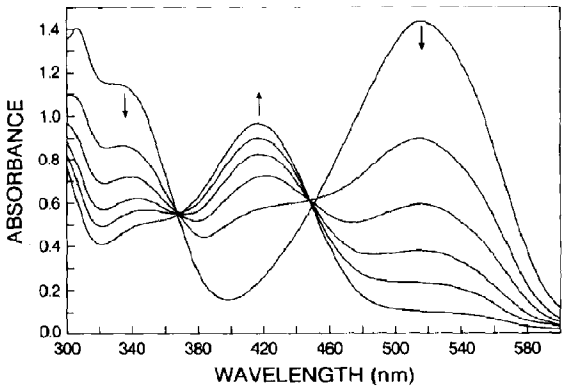


Fig. 5. Ultraviolet-visible absorption spectra from deaerated solution (pH 7.1) containing AM1 (0.133 mM), and NADH (0.166 mM) upon illumination ($\lambda > 475$ nm) for 0, 15, 30, 45, 60, and 90 s. Arrows indicate direction of changes. From ref. 42.

as NADH, was necessary to induce the DNA ssb with AM1, it markedly enhanced the effect with AM2 (fig. 6) [43]. Using oxygen consumption as an indicator of the photosensitized oxidation of NADH, it was established that upon binding of the drug to DNA, it loses its sensitizing properties, (fig. 7) [43]. Only agents free in solution were able to photosensitize the oxidation of NADH. In addition, correlations between the extent of DNA degradation, oxygen consumption and NADH oxidation were established [43].

Anthracenedione agents have been tested for their ability to photosensitize neoplastic cells in culture [44]. In the case of AM1 and AM2 light illumination (which was completely non-toxic to cells in the absence of drugs) significantly shifted the viability curves of human leukemic cells (fig. 8). In contrast, AM and MX gave no significant light-induced dose modification under comparable conditions. Sites of photodamage were also assessed [44]. No significant membrane damage could be detected as determined by transport of the model amino acid cycloleucine. This result is in contrast to porphyrins which photosensitize mainly at sites of amino acid and nucleoside transport [45]. DNA single-strand breaks as measured by the technique of alkaline elution, however, did correlate well with cell viability even though localization of the drug intracellularly,

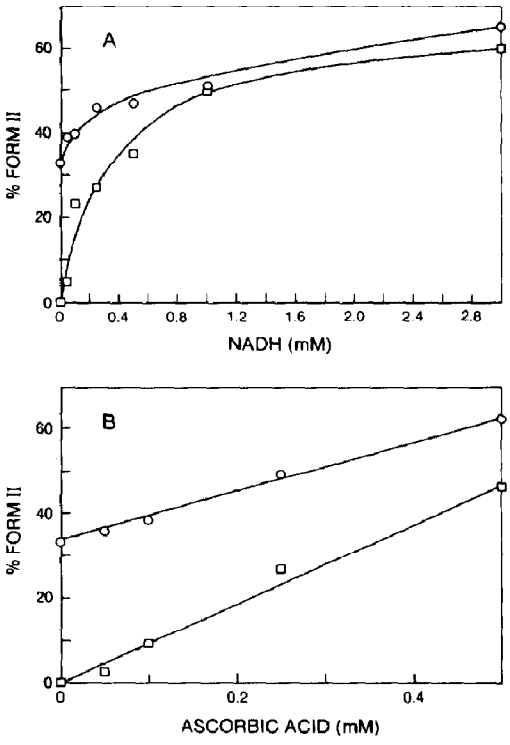


Fig. 6. The effect of concentration of NADH (A) or ascorbic acid (B) on the photoactivated cleavage of PM2 DNA by AM1 (□) and AM2 (○). Samples at drug/DNA base-pair ratio of 1 (20 μM final concentrations) were illuminated for 8 min and the percent of form II DNA determined by microdensitometry of the negative produced upon photography of the agarose gel. From ref. 43.

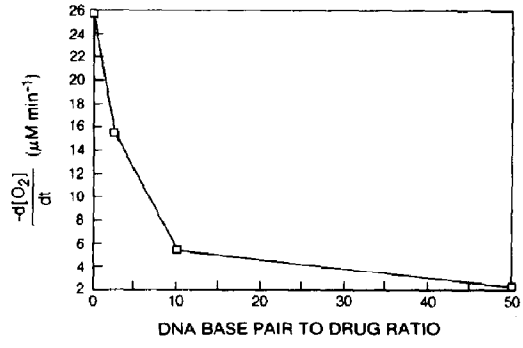


Fig. 7. The effect of increasing DNA concentration on the rate of oxygen consumption in illuminated samples of AM2 in the presence of NADH. The AM2 concentration remained constant at 8.5 μM throughout. From ref. 43.

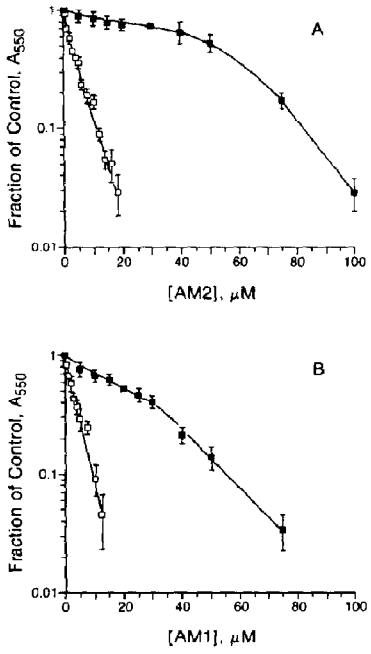


Fig. 8. Cell viability curves for AM2 (A) and AM1 (B) in the dark (■) or following 2 min of illumination (□) at $\lambda > 475$ nm. From ref. 44.

using fluorescence microscopy, indicated that the majority of the drug was located in the cytoplasm and not in the nucleus.

There is a large body of data, collected during a period of more than 40 years, concerning various photophysical/photochemical characteristics of many diamino substituted anthraquinone dyes due to their widespread industrial applications. These compounds have chromophores identical, or very similar, to the agents shown in fig. 1. Therefore, photophysical parameters and photosensitizing properties of the anthracenedione anticancer agents should be similar to those established previously for the dyes mentioned above. These data can be a useful source of reference, permitting a correlation between the sensitizing properties of these two groups of compounds.

Thus, the longest wavelength bands of the aminoanthraquinones, $\pi \rightarrow \pi^*$, were assigned to the intramolecular charge-transfer transitions from the amino to the carbonyl groups [46–48]. The exact position of these absorption bands depends on the electron-donating capability of the substituent [46–51]. Mono- and disubstituted aminoanthraquinones fluoresce with quantum yields of less than 0.27, and in most cases the yield is much lower, depending on the type and position of the amino substituent in the chromophore and solvent [52–55]. Radiative lifetimes of the first excited singlet states of these anthraquinones in acetonitrile were determined to be approx. 1 ns or less [54]. Rate constants of the fluorescence quenching by a series of anilines and methoxybenzenes in acetonitrile were measured and correlated with the redox properties of electron donors (quenchers) and acceptors (aminoanthraquinones) and free enthalpy changes [54].

Quantum yields of triplet state (T_1) formation, for a series of 1,4-diaminoanthraquinones (1,4-DAA) in benzene have been determined using laser flash photolysis [56]. The ϕ_T values determined using anthracene as a standard were less than 0.029 [56]. First-order decay rate constants of these triplets in the same solvent are in the range of $1.0–1.8 \times 10^5$ s^{-1} [56]. Similar values were obtained employing pulse radiolysis methods [57]. It was suggested that the very short lifetimes of the triplets from 1,4-DAA is a consequence of the quenching by reversible hydrogen abstraction across the intramolecular and/or intermolecular hydrogen bonds [57]. Energies of the T_1 triplet states were estimated to be in the range of 97–151 kcal mol^{-1} for these anthraquinones [57]. The results of this study also suggest that these dyes dimerize in the ground state in benzene solutions, even at low concentrations [57].

In contrast to 1,4-DAA, 1,5-DAA and 1,8-DAA show much higher ϕ_T values. For example, ϕ_T values of 0.67 and 0.77 reported respectively for the 1,5-DAA and 1,8-DAA isomers in chloroform [58]. The ϕ_T values may differ depending on the method applied and the solvent used, however they are consistently much higher (at least 30 times) than for the corresponding 1,4 isomers [59]. Rate constants of the triplet decay for 1,5-DAA and 1,8-DAA are 10^5 s^{-1} [58], i.e., they are of the same order as those found for the 1,4-DAAs. Therefore, it may be concluded that the dif-

ferences in reactivities among various isomers of this class of compounds are related to yields of formation of the photoactive triplet states. Possible reasons for such significant variations in ϕ_T among the mono- and disubstituted aminoanthraquinones have been discussed [59]. In aminoanthraquinones the lowest triplet state is of the $\pi\pi^*$ type and is characterized by poor reactivity. It has been inferred that in the case of 1,5-DAA, the state that is the most photochemically reactive is $T_2(n\pi^*)$, which may be populated via thermally assisted intersystem crossing from the singlet state, $S_1(\pi\pi^*)$, owing to the small energy gap between these two states. It is possible that a similar mechanism operates in the case of 1,8-DAA. This route is inaccessible, however, for the 1,4-DAAs, because in this case the energy difference between the $S_1(\pi\pi^*)$ and $T_2(n\pi^*)$ states is too high and $E(T_2) \gg E(S_1)$ [59].

Although 1,4-DAAs cannot be photoactivated with visible light, this can be accomplished using ultraviolet irradiation, which could lead to the population of the upper triplet state, T_2. *N,N'*-Dimethyl-1,4-diaminoanthraquinone was readily reduced in ethanol solution upon irradiation in the wavelength region (250–350 nm) of the $n\pi^*$ band, while no evidence of a photochemical reaction was found on illumination at the long-wave absorption band [60]. Similarly, mitoxantrone could be activated only upon ultraviolet irradiation (313 nm) and then caused the decarboxylation of peptides [41]. A similar mechanism of photoactivation was described for 1-aminoanthraquinone which undergoes photoreduction via the upper excited $T_2(n\pi^*)$ level [61].

The differences in photochemical reactivities between the various structural isomers of diaminoanthraquinones were further confirmed in studies on singlet oxygen generation (reaction of type II). Again 1,5- and 1,8-DAAs and even 1,5-diamino-4,8-dihydroxyanthraquinone sensitized formation of singlet oxygen in organic solvents upon illumination with visible light, while the 1,4-DAAs appeared to be inactive [58,62,63]. It may be concluded that the photosensitizing properties of the anticancer agents MX, AM, and AM1–AM4, parallel those found for other diaminoanthraquinone dyes.

4. Anthracyclines

Certain toxic side effects observed in patients receiving daunorubicin (DA) treatment were linked to the drug photoactivity [64,65]. In fact, as early as 1968 it was demonstrated that visible light illumination of DA can kill viruses and bacteria in vitro [66,67]. This phototoxic effect may be related to DA-photoinduced DNA degradation [68]. Formation of covalently bound adducts of DA and DX (doxorubicin) with DNA upon ultraviolet and visible light treatment was also reported [69,70]. On the basis of EPR and spin-trapping studies, which showed formation of DMPO-OH species, it was suggested that OH radicals are produced upon DA illumination, and consequently could be involved in the DNA photodegradation process [68]. However, control experiments conducted in the presence of OH scavengers (ethanol, DMSO) did not confirm production of OH radicals in such a system [71,72] and therefore, the DMPO-OH adduct observed must have another origin [73,74]. The mechanism of formation of the DMPO-OH radical may be affected by experimental conditions, such as the presence of oxygen, concentration of drug or spin trap, or the applied excitation wavelength. Recent EPR studies employing $^{17}O_2$ and $H_2{}^{17}O$, indicated, however, that both oxygen gas and water molecules contribute to the formation of the DMPO-OH adduct during aerobic DA photolysis, while anaerobic photolysis involves interaction with water molecules only [75]. Participation of superoxide radical in this process has been excluded as a possibility.

Production of superoxide radical upon visible light illumination of DX in DMSO [75] and in aqueous solution [71] was reported by some authors while other workers observed SOD-sensitive generation of the superoxide-DMPO adduct only upon ultraviolet (310 nm) activation [72].

Two different kinds of carbon-centered radicals were detected as DMPO adducts, upon ultraviolet irradiation (310 nm) of DA and DX in deaerated solutions [72]. They were assigned to the drug-derived alkyl and acyl radicals. Anaerobic photolysis of DA and DX at 310 nm, in the absence of DMPO, produced EPR spectra of the semiquinone radicals from those drugs [72].

Decarboxylation of peptides, specifically at the C-terminal amino acid of the peptide, by the photoactivated (313 nm) anthracycline drugs was reported [41]. In air-free solutions nucleic acid bases are oxidized in the presence of DX when irradiated at 310 nm, yielding C(5)-carbon-centered radicals in the pyrimidine rings of uracil, cytosine and thymine [41]. Spin adducts of MNP with radicals at the N(1) or N(2) positions of the pyrimidine ring were also detected [41].

Naphthazarin and quinizarin resemble the quinone-hydroquinone moiety of the anthracycline antibiotics and therefore serve as convenient model compounds for studying the pathways of generation and the properties of their free radical forms. The EPR spectrum of the naphthazarin-derived radical was readily produced by ultraviolet photolysis, ionizing radiation, electrolysis or chemical reduction using ascorbic acid [76,77]. Radicals from quinizarin were readily produced by electron or ultraviolet irradiation [76] and chemical reduction [78]. These radicals were stable in deoxygenated aqueous solutions but decayed rapidly in the presence of air. Their stability also depends on the pH of the medium. An important role in the stabilization of these radicals, which may be relevant to the biological activity of anthracyclines, was ascribed to the formation of intramolecular hydrogen bonds [76,77].

Photochemical reactivity of DA has been further confirmed in experiments employing NAD(P)H as electron donor [79]. Brief illumination of the drug and NADH in a deaerated DMF/buffer (pH 7.4) mixture (1 : 1, v/v) with blue light (approx. 480 nm) of low intensity (approx. 10 W/m^2), generates a strong EPR signal with well-resolved high-frequency structure, assigned to the radical anion of DA. In the absence of oxygen, the radicals decayed according to second-order kinetics, most likely in a disproportionation reaction with the rate constant of 5.15×10^2 M^{-1} s^{-1} [79]. In aqueous buffer the rate constant was two orders of magnitude higher [79]. A similar value was obtained from the decay kinetics of enzymatically produced DA radicals [30]. However, a pulse radiolysis study reports a significantly higher value for the rate constant, i.e., approx. 10^8 M^{-1} s^{-1} [80].

The superoxide radical was readily produced upon illumination of aerated solutions of DA/NAD(P)H/DMPO in DMF/buffer, pH 7.4 (1 : 1, v/v) [79]. The EPR spectrum of the DMPO-O_2^- adduct was sensitive to the presence of SOD. The yield of radical production was significantly higher in the DMF/H_2O mixture than in aqueous solution (pH 7.4). The facilitation of these photochemical reactions in DMF/H_2O may be related to the fact that in this solvent the drug exists in a non- or less-aggregated form than in aqueous solution which may affect its photosensitizing capabilities. This observation may be of relevance to the anthracycline-induced redox cycling reactions which take place in hydrophobic regions of the cell membrane. The role played by solvent composition in the EPR spectra of the drug-derived radicals produced in enzymatic reactions, has been examined in ref. 81. 5-Iminodaunorubicin, in contrast to its parent compound, DA, is devoid of photosensitizing activity [79].

Short-lived, oxygen-sensitive, transient species from DA and DX were detected in aqueous solutions (phosphate buffer, pH 7.4) after the laser flash and were ascribed to the triplet states of DA and DX [82,83]. The transients decayed with a first-order rate constant of 10^5 s^{-1}. It was estimated that for DA and DX, ϕ_T is approx. 0.2 in aqueous solutions, and is close to the ϕ_T value for (daunomycinone) in benzene [82,83]. Fluorescence quenching experiments with I^- resulted in an excited singlet state quenching constant of approx. 2×10^{10} M^{-1} s^{-1} [82,83]. Extremely weak luminescence at 1270 nm, characteristic of singlet oxygen phosphorescence, was recorded from the ^2H_2O solutions of DA and DX upon a laser flash [82,83]. The singlet oxygen quantum yield, ϕ_Δ, was estimated to be near 0.02. The second-order rate constant for the singlet oxygen quenching by DA and DX in aqueous solutions was determined as approx. 1×10^7 M^{-1} s^{-1} [82,83]. Yields of singlet oxygen production and quenching may be affected by the degree of aggregation of the drugs.

5. Conclusions

A number of new anticancer agents, from the class of chromophore-modified anthracycline ana-

logs, proved to possess strong photosensitizing capabilities.

Deshydroxyanthrapyrazoles and 1,5- and 1,8-diamino-substituted anthraquinones can oxidize biochemical electron donors (NAD(P)H, catechols, ascorbic acid), and induce DNA damage and cell killing upon activation with visible light. Photochemically produced drug-derived radicals can undergo redox cycling with oxygen, forming superoxide and hydrogen peroxide, or react with hydroperoxides, forming hydroxyl or alkoxy radicals, and therefore play an important role in the development of light-induced damage to cell constituents.

There is an apparent correlation between the photosensitizing properties of anthrapyrazoles and their structural characteristics, such as the position and number of hydroxyl groups in the chromophore, capable of forming intramolecular hydrogen bonds. The strongest photosensitizer (AP1) possesses only one such bond. Anthracenediones also form intramolecular hydrogen bonds, however only the 1,4 isomers (MX and AM) are devoid of photosensitizing properties. Similarly, photoactive anthracyclines (DX and DA) can also form such bonds. Therefore, the existence of intramolecular hydrogen bonds in a chromophore is not sufficient to preclude the possibility of photoreactivity of this compound and other factors must be considered instead. For example, symmetry of the molecule and composition of the medium can play important roles in this respect.

The fact that these new agents also show low dark toxicity and dark anticancer activity makes them particularly interesting as potential agents for PDT and therefore warrants further studies. At present, work is in progress on the synthesis of anthrapyrazole and anthracenedione derivatives with red-shifted absorption characteristics, and establishing the degree of phototoxicity of these novel agents in vitro using cell aggregates, i.e., spheroids, as model systems for access to cells of varying degrees of anoxia.

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