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Review

Photooxygenation, Photodegradation and Antioxidative Activity of Platonin, a Cyanine Photosensitizing Dye

MARIKO ISHIHARA and SEIICHIRO FUJISAWA

Meikai University School of Dentistry, Saitama 350-0283, Japan

Abstract. *Platonin (4,4'-dimethyl-3,3'-di-n-heptyl-8-[2-(4-methyl-3-n-heptylthiazole)] -2,2'-dicarbocyanine diiodine) is one of the photosensitive trithiazolepentamethine cyanine dyes. Visible light (VL)-promoted photodegradation products of platonin in an aqueous environment were identified as 3-heptyl-4-methylthiazoline-2 -carbaldehyde (**1**), tetradecane-7-thiol (**2**), 1-nonene (**3**), heptylamine (**4**), 3-heptyl-4-methyl-2-thiazolone (**5**), 3-heptyl-4-methyl-2-thiothiazolone (**6**), 5-[2-(3-heptyl-4-methylthiazolidene)]-2-penten-1-aldehyde (**7**), γ-(3-heptyl-4-methyl-2-thiazolidene)crotonic acid (**8**) and 3,5-di (4-methyl-2-thiazolyl)-2,4-pentadienic acid (**9**). The quantum yield of singlet oxygen ($^1O_2$ ($^1\Delta_g$)) derived from VL-promoted platonin formed heptyl and heptyl cation radicals together with the photodegradation products described above. In isolated rat hepatocytes, platonin was cytotoxic under VL irradiation, whereas non-irradiated platonin was less cytotoxic and improved cell viability. The effects of oxygen uptake and cell viability of photolysis photoproducts of platonin, 3-heptyl-2,4-dimethylthiazolium iodide (HDT) and 3-heptyl-4-methylthiazolium iodide (HMT) were compared with those of platonin. These compounds, particularly the former, showed greater cytotoxicity and brought about less oxygen uptake than the latter. Radical-scavenging activities of platonin using an induction period method demonstrated that fully oxidized platonin had a stoichiometric factor (n) of 4. Platonin was a potent peroxy-radical scavenger. The dual modulation activity of platonin as a prooxidant and an antioxidant under VL irradiation was revealed by monitoring the oxygen uptake in isolated rat hepatocytes. This antioxidant/prooxidant activity of platonin may induce diverse effective pharmacological activities in biological systems. In the light of recent developments in studies of platonin and related compounds, the VL-promoted photooxygenation, photodegradation, antioxidant activity and biological activity of platonin are discussed.*

Correspondence to: Dr. M. Ishihara, Division of Chemistry, Department of Oral Biology and Tissue Engineering, Meikai University School of Dentistry, Sakado, Saitama 350-0283, Japan. Tel/Fax: +81 492 85 5511, e-mail: mariko@dent.meikai.ac.jp

Key Words: Platonin, visible light, antioxidants, prooxidant, photodegradation, oxygen uptake, biological activities, review.

Platonin, a cyanine photosensitizing dye, has been reported to have antimicrobial activities (1-3), pharmacological activities such as burn-healing promotion (4), and antihistaminic (5) and anticancer activity (6). Platonin pretreatment has been shown to suppress acute inflammation (7). Platonin had also effects in rat models of endotoxemia which may have been mediated by a reduction of mean arterial blood pressure and inhibition of NO and free radical formation (8). Platonin inhibited the production of pyrogenic cytokines from human peripheral blood mononuclear cells (PBMC), resulting in antipyresis. Lipopolysaccharide (LPS) was shown to induce NF-kappaB activation of PBMC, and this effect was abolished by platonin (9). Platonin attenuated NO production and *L*-arginine transport in LPS-stimulated murine macrophages, possibly through inhibition of inducible NO synthase (iNOS) and cationic amino-acid transporter (CAT-2 and CAT-2B) expression (10). Platonin is thus a potent macrophage-activating agent and immunomodulator. However, there have been few studies on the photochemical properties of platonin (11), although this area of investigation is of great relevance from a biological as well as medical viewpoint. Here, the results of our studies in this area are described, and the photooxygenation, photodegradation and antioxidative activity, and biological properties of platonin are discussed.

Photooxygenation

Singlet oxygen and oxygen uptake. Photosensitization reactions are generally considered as belonging to either type I (radical mediated) or type II (singlet oxygen mediated) (12, 13). The mechanism of photooxygenation reactions sensitized by dyes, such as rose bengal, eosin and methylene blue, belongs to type II (13).

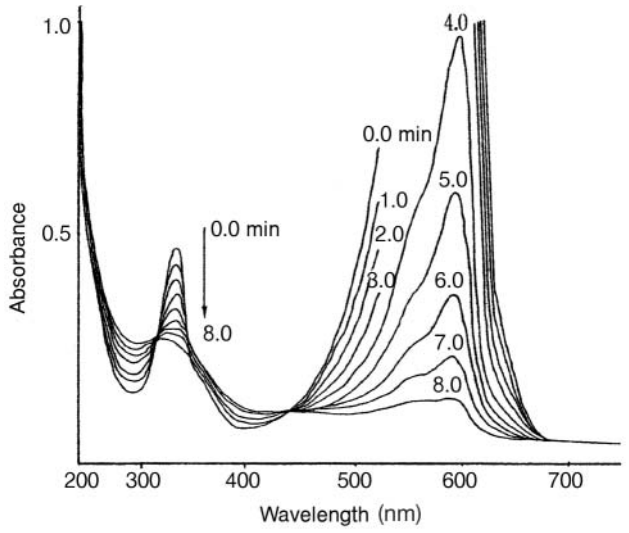

Figure 1. *Changes in absorption curves of platonin during VL-irradiation. Irradiation time (min) is shown in the inset.*

Table I. *Effects of visible light (VL)-irradiated platonin on oxygen uptake and viability of rat hepatocytes after 2 h incubation.*

Irradiation time (min)	Oxygen uptake (%)	Cell viability (%)
0	153.5	104.5
5	97.8	99.9
10	88.9	103.7
20	93.0	101.2

Values are expressed as percentage of control. Platonin $1.5x10^{-4}$ M. The data are cited from the literature (11).

Platonin is a cell invigorator of the trinucleustetramethine cyanine dye type and is unstable upon exposure to light, showing momentary fading after irradiation. Photooxygenation of platonin possibly occurs in a manner similar to that of rose bengal, eosin and methylene blue (14, 15). Photosensitized oxygenation of platonin produces quantum yields of singlet oxygen ($^1O_2$) as shown below (Type II):

$$^3\text{Platonin} + {}^3O_2 \rightarrow \text{platonin} + {}^1O_2 \quad [1]$$

$$A + {}^1O_2 \rightarrow AO_2 \quad [2]$$

where ^{3}platonin acts as a sensitizer in a triplet excited state, A is a substrate (or other compounds present in the reaction medium), such as olefins, amines, or organosulfur compounds, and AO_2 is the oxygenation product.

Quenching of the singlet and triplet excited states of platonin is caused by molecular oxygen ($^3O_2$). Singlet oxygen, O_2 ($^1\Delta_g$), yields an oxygenation product (AO_2). $^1O_2$ further reacts with electron-rich olefins by mean of the ene reaction, and the 1,4-and 1,2 addition reaction, and also participates in the oxidation of organosulfur compounds (16). Platonin in aqueous solution is much less stable than in organic solvents. It degrades easily in water and in air, resulting in fading. The changes in the spectral absorption curves of platonin in water are shown in Figure 1. The absorption bands (λ_{max}=330 and 588 nm) show a decrease with increasing length of VL-irradiation.

Table I shows oxygen uptake for VL-irradiated platonin in isolated hepatocytes. Platonin was irradiated for 5, 10 or 20 min prior to addition of hepatocytes whose oxygen uptake was then investigated. It was of interest that platonin-treated hepatocytes were in a much more fully energized state than VL-irradiated platonin-treated cells, as shown by their greater oxygen uptake. The relationship between oxygen uptake and reaction time for platonin-treated cells in the absence (no irradiation) or presence (irradiation) of VL irradiation and for platonin-untreated cells (no dye, controls) is shown in Figure 2. The plots for the unirradiated and control groups gave linear curves, and the former showed greater oxygen uptake than the latter. In contrast, the curve for oxygen uptake of platonin under VL irradiation was virtually identical to that of the control for approximately 1 h, but thereafter showed a considerable divergence. After 2 h, oxygen uptake had declined by about 30% compared to cell treated with un-irradiated platonin, followed by a decrease in cell viability of (data not shown) about 20%.

These findings may be attributed to a dual antioxidant and prooxidant modulation activity of platonin. Platonin under VL irradiation possibly generates singlet oxygen and the related superoxide anion, reactive oxygene species (ROS) ($^1O_2$, O_2^-, OH^-), in isolated hepatocyte suspensions, but when platonin is present in excess, it preferentially scavenges ROS, acting as an antioxidant in this process. As the irradiation time extended beyond 1 h under these experimental conditions, platonin was considerably degraded due to the fact that it scavenged molecular oxygen as a biradical, and consequently degradation products were formed, platonin thus acting as a prooxidant in this process. Thus, the intrinsic cell energetics of platonin are suppressed by intensive VL irradiation, possibly due to ROS formation. A possible link between cell viability and oxygen uptake for platonin was suggested. Platonin alone was less cytotoxic and also improved cell viability (Table I), whereas under VL irradiation platonin became highly cytotoxic; incubation with VL-preirradiated platonin was less cytotoxic.

Platonin produces highly cytotoxic singlet oxygen by collisional energy transfer from the excited triplet state of the dye to the ground triplet state of oxygen. In addition, an alternative pathway involves the formation of free radicals – the heptyl radical and the heptyl cation radical – from platonin through either cleavage of specific bonds in the dye or

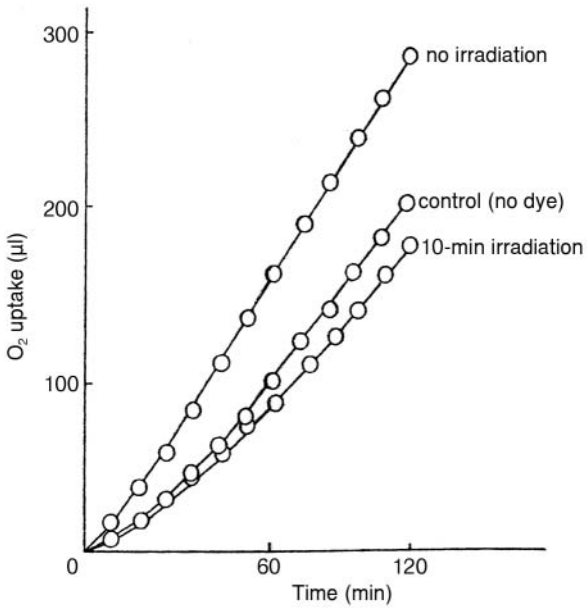

Figure 2. *Plots of oxygen uptake vs VL-irradiation time for irradiation, no irradiation and control (no dye). No irradiation: platonin in dark conditions without VL-irradiation; control: no platonin; irradiation: platonin under VL-irradiation. Platonin: 1.32x10^{-4} M. A 0.5-ml aliquot of sample solution was added to a suspension of isolated rat hepatocytes (3 ml) in a plastic reactor vessel. After preincubation of the mixture at 37°C for 3 min, oxygen uptake was monitored continuously for 2 h using an oxygen sensor; irradiation was done using a 150W lamp. The vessel contained 2.5-3.0x10^6 hepatocytes in Krebs-Ringer phosphate buffer containing 0.1% glucose.*

electron transfer processes (see next section). Uptake of platonin from buffer solution into the mitochondrial matrix possibly occurs in suspensions of isolated rat hepatocytes. Mitochondrial photosensitization is of interest because mitochondria play a key role in the oxidation processes involved in cell metabolism, as well as in the regulation of normal cell functions. Platonin acts as an uncoupler of oxidative phosphorylation in mitochondria (17) due to its action on adenine nucleotide translocation, rather than by electrophoretic transfer into the inner space of the mitochondrion in accordance with the inside-negative electrochemical potential (18). The reaction of platonin-mediated ROS and free radicals with unsaturated lipids and proteins in the membranes may directly cause alteration of membrane functions. The photosensitization of cell membranes with dyes has been shown to decrease the membrane potential, inhibit the transmembrane transport of metabolites, and both inhibit and activate membrane-associated enzymes (19). Alterations of mitochondria by platonin under VL irradiation may cause perturbation of cellular structure and severe cell damage if platonin concentrations and VL-irradiation times are not carefully controlled. The cellular functions of platonin under VL irradiation may be analogous to oxygen uptake by *tert*-butyl hydroperoxide (TBH) (20). Isolated digitonin-permeabilized rat hepatocytes were used to evaluate the toxic effects of TBH on the function of the mitochondrial enzyme complex (20). TBH selectively inhibits mitochondrial respiratory- chain enzymes in isolated hepatocytes and greatly suppresses oxygen uptake. In this system, endogenous respiration (oxygen uptake) is significantly increased by addition of the uncoupler dinitrophenol and decreased by rotenone. Two mechanisms for the action of TBH have been proposed: oxidation of the functionally important SH-group in mitochondrial enzymes (21) and/or changes in mitochondrial membrane integrity induced by peroxidation of membrane lipids (22). Platonin, a trinuclear divalent cationic cyanine dye, is expected to act as a radical ion when it absorbs light and changes to the triplet state (11). If this radical ion decomposes, the reaction mechanism may be similar to that of TBH described above. Platonin under VL irradiation may inhibit mitochondrial respiratory- chain enzymes. In contrast, platonin alone enhances oxygen uptake, possibly due to the uncoupling of oxidative phosphorylation (17) in a manner similar to dinitrophenol. Compounds such as 3,4,5,6-tetrachloro-2-trifluoromethylbenzimidazole (TTMB), carbonylcyanide-*p*-trifluoromethoxy phenylhydrazone (FCCP) and carbonylcyanide phenylhydrazone (CCP), bearing cyanide groups as does platonin, are well-known uncouplers.

The formation of intracellular ROS induced by VL-excited photosensitizing dyes, and photoinitiators for resin polymerization such as 9-fluorenone (9F) and camphorquinone (CQ) for curing methacrylates has been previously reported (23). VL-irradiated 9F and CQ had a strongly cytotoxic effect under VL irradiation, particularly 9F, possibly due to ROS formation. In addition, cancer cells treated with curcumin (a component of the spice turmeric) accompanied by VL-irradiation showed strong formation of intracellular ROS, accompanied by induction of apoptosis/necrosis (24). Oxidative stress induced by VL-promoted photodegradation of platonin *via* singlet oxygen may thus trigger necrosis and apoptosis. Moreover, ROS may act as regulatory factors of cell metabolites as well as participating in necrosis and apoptosis. VL-excited methylene blue causes oxidative DNA damage in primary rat cells, possibly due to the formation of singlet oxygen (25). VL-excited platonin may cause DNA damage, but few studies have investigated the induction of apoptosis by platonin.

Photodegradation

Degradation products. The photodegradation products of platonin in aqueous solution after 3 h exposure to VL-irradiation (from a 500 W photoreflector lamp with a filtered light frequency below 450 nm) were determined using NMR

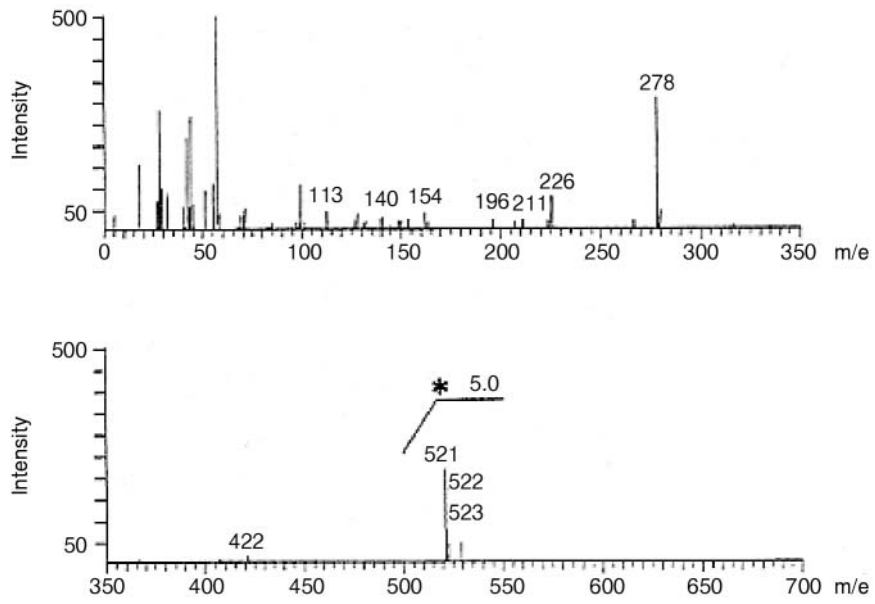

Figure 3. *Mass spectrum of platonin.*

spectroscopy, gas chromatography-mass spectrometry (GC-MS) and/or thin-layer chromatography (11). The degradation always operated *via* singlet molecular oxygen, forming a heptyl radical and heptyl cation radical due to ring-opening of the thiozoline moiety and cleavage of the conjugated system in the platonin molecule. This was strongly supported by experiments investigating the decomposition of platonin in the bombardment chamber of a mass spectrometer, which demonstrated marked fragment peaks at m/z 523, 522, 521, 422, 278, 226, 211, 196, 154, 140 and 113 (see the mass spectrum of platonin in Figure 3). The fragmentations are shown in Figures 4A and B, and the photoproducts were identified (Figure 5). From these findings it is reasonable to propose a mechanism for the photodegradation of platonin (Figures 5A and B). Briefly, photoproduct **5** would be formed by cycloreversion of dioxetane, resulting in the [2+2]cycloaddition of platonin with singlet oxygen. In fact, the possibility of formation of dioxetanes through [2+2]cycloaddition of singlet oxygen to olefins has been suggested previously (12) and dioxetane was stably obtained (26). Photoproduct **7** was formed through decarboxylation of carboxylic acid produced by hydrolysis of the thiazole ring of one of the parts remaining from the formation of **5**. In addition, photoproduct **9** would have been formed through the release of two heptyl groups and oxidation of the aldehyde group of one of the parts remaining from the formation of **5**. Photoproduct **1** was probably formed as follows: the position-3 dioxetane bond, produced through the [2+2] cycloaddition of singlet oxygen, would be decomposed and cleavage of the O-O bond would probably occur, forming photoproduct **1**. Photoproduct **8** was formed *via* hydrolysis and decarboxylation of the thiazole ring of one of the parts remaining from the formation of photoproduct **1**. Photoproduct **6** would be formed as follows: the position-5 bond at the terminal thiazolium ring would be hydrolyzed, and the hydrolysate bond at position-2 of the central thiazole ring would become the first intermediate, followed by cycloreversion to yield **6**. The *n*-heptyl cation or *n*-heptyl radical probably reacted with the sulfur of the thiazoline ring or its hydrolysis product, and then another *n*-heptyl radical would have attacked the α-position of the first heptyl group attached to the sulfur of the active methylene group. Hydrolysis of this intermediate would then yield photoproduct **2**. Photoproduct **3** was probably formed through decomposition followed by heptyl radical attack on the methylene group of platonin. Simple hydrolysis of the thiazoline ring would yield photoproduct **4**. That is, singlet oxygen molecule- promoted degradation of platonin would yield photoproducts **1-9**, together with the heptyl and heptyl cation radicals.

The biological activities of platonin under VL irradiation were clearly affected by singlet molecule oxygen- promoted degradation products together with the formation of free radicals. Having discussed the catalytic and prooxidative properties of platonin, we now focus on the antioxidative properties of platonin.

Radical-scavenging Activity

Stoichiometric factors (n) and inhibition rate. Platonin possesses antioxidative activity. Photosensitizing compounds such as platonin scavenge lipid radicals derived from auto-oxidation of methyl linoleate (27) and exert an antiperoxidant

Table II. *Comparative radical-scavenging activity of platonin and other antioxidants.*

Compound	*n*	Rp_{inh}/Rp_{con}	References
Platonin	3.8	0.4	(35)
Melatonin	0.4	0.4	(33)
Ebselen	0.1	1	(34)
BHA	2	1	(30)
BHT	1.1	1	(30)
N-Methyaniline	0.8	0.8	(31)
Catechin	3.4	0.7	(32)

n, stoichiometric factor; Rp_{inh} and Rp_{con} are the initial rate of polymerization in the presence and absence of antioxidants, respectively. The procedures are described in the text.

Table III. *Effects of platonin, HMT and HDT on oxygen uptake on viability of hepatocytes after 2 h incubation.*

Drug	Concentration (M)	Oxygen uptake	Cell viability
HMT	$2.0x10^{-5}$	100.0	102.5
	$1.0x10^{-4}$	90.0	102.0
	$2.0x10^{-4}$	56.3	108.8
	$1.0x10^{-3}$	27.7	57.4
HDT	$2.0x10^{-5}$	96.8	100.2
	$2.0x10^{-4}$	85.4	89.7
	$5.0x10^{-4}$	24.3	60.1
	$1.0x10^{-3}$	7.9	23.4
Platonin	$1.0x10^{-3}$	99.4	100.8
	$1.0x10^{-2}$	98.1	100.0

Values are expressed as percentage of controls. Data were cited from the literature (37).

action on the lipids in biomembranes (28). Furthermore, platonin reduces the ESR signal intensity of the superoxide anion, hydroxy radical, and methyl radical formation in the H_2O_2/NaOH/DMSO system (8). Platonin possesses the capacity not only to scavenge free radicals but also to reduce plasma nitric oxide (NO) formation during sepsis (8). Although platonin possesses antioxidant activity, the kinetics of its reaction with carbon-centered or oxygen-centered radicals under anaerobic conditions remain unknown. For maximum biological relevance, studies on platonin should be performed under anaerobic conditions, because biological systems have a low oxygen tension (29).

We previously proposed a quantitative model rationalizing the radical-scavenging activity of butyrated hydroxytoluene-related compounds (30), polyamines (31), polyphenols (32), melatonin (33), ebselen (34) and platonin (35) in polymerization of methyl methacrylate (MMA) initiated by thermal decomposition of azobisisobutyronitrile (AIBN) or benzoyl peroxide (BPO) under nearly anaerobic conditions. The model was well able to explain the mechanism of the radical-scavenging activity of bioactive compounds (30-35).

The radical-scavenging activities of platonin were investigated by the induction period method in the polymerization of MMA, initiated by thermal decomposition of BPO (an oxygen-centered radical, PhCOO·), under nearly anaerobic conditions. The *n* value was determined using Equation **3**.

$$n = R_i[IP]/[IH] \quad [3]$$

where [IP] is the induction period in the presence of an inhibitor, and R_i is the rate of decomposition of the initiator. The R_i value of BPO was $2.28x10^{-6}$ Ms^{-1} (11). When polymerization is suppressed and retarded by an inhibitor, the rate can be expressed by Equation **4**.

$$Rp_{inh}/Rp_{con}=(2k_tR_i)^{1/2}/\{n\ k_{inh}[IH]\} \quad [4]$$

where Rp_{inh} and Rp_{con} are the initial rates of polymerization in the presence and absence of an initiator, respectively. k_t and k_{inh} are the rate constants of termination and inhibition, respectively.

Table II shows the *n* and Rp_{inh}/Rp_{con} values for platonin, melatonin, catechin, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), *N*-methylaniline, and ebselen. The *n*, *i.e.* (the number of moles of radicals trapped by platonin calculated with respect to 1 mole of inhibitor moiety unit) for platonin was approximately 4 and was similar to that of catechin and greater than that of conventional phenolic synthetic antioxidants (BHA and BHT), methylaniline or ebselen. Based on the Rp_{inh}/Rp_{con} value, the k_{inh} value was calculated and the value for platonin was compared with those for other antioxidants. The k_{inh} of platonin determined using Equation **4** with $k_t=3.7x10^7$ $mol^{-1}s^{-1}$ (31) was $1.71x10^3$ $M^{-1}s^{-1}$. This value was similar to that of BHA, BHT and catechin, indicating that platonin was as potent in terms of radical-scavenging activity.

To elucidate the interaction between platonin and thiols, 2-mercapto-1-methylimidazole (MMI) was used as a representative for thiols, because gluthathione use was unsuccessful due to limited solubility in MMA. MMI in the presence of platonin showed neither catalytic activities nor synergistic activities (35).

Biological Activities

Biological effects of thiazole derivatives. Some of photodegradation products of platonin may possess biological properties. Platonin yields 3-heptyl-2, 4-dimethylthazolium (HDT) and 3-heptyl-4-methylthiazolium (HMT) upon photolysis (36). Oxygen uptake and cell viability of hepatocytes in the presence of HDT and HMT were investigated and results are summarized in Table III.

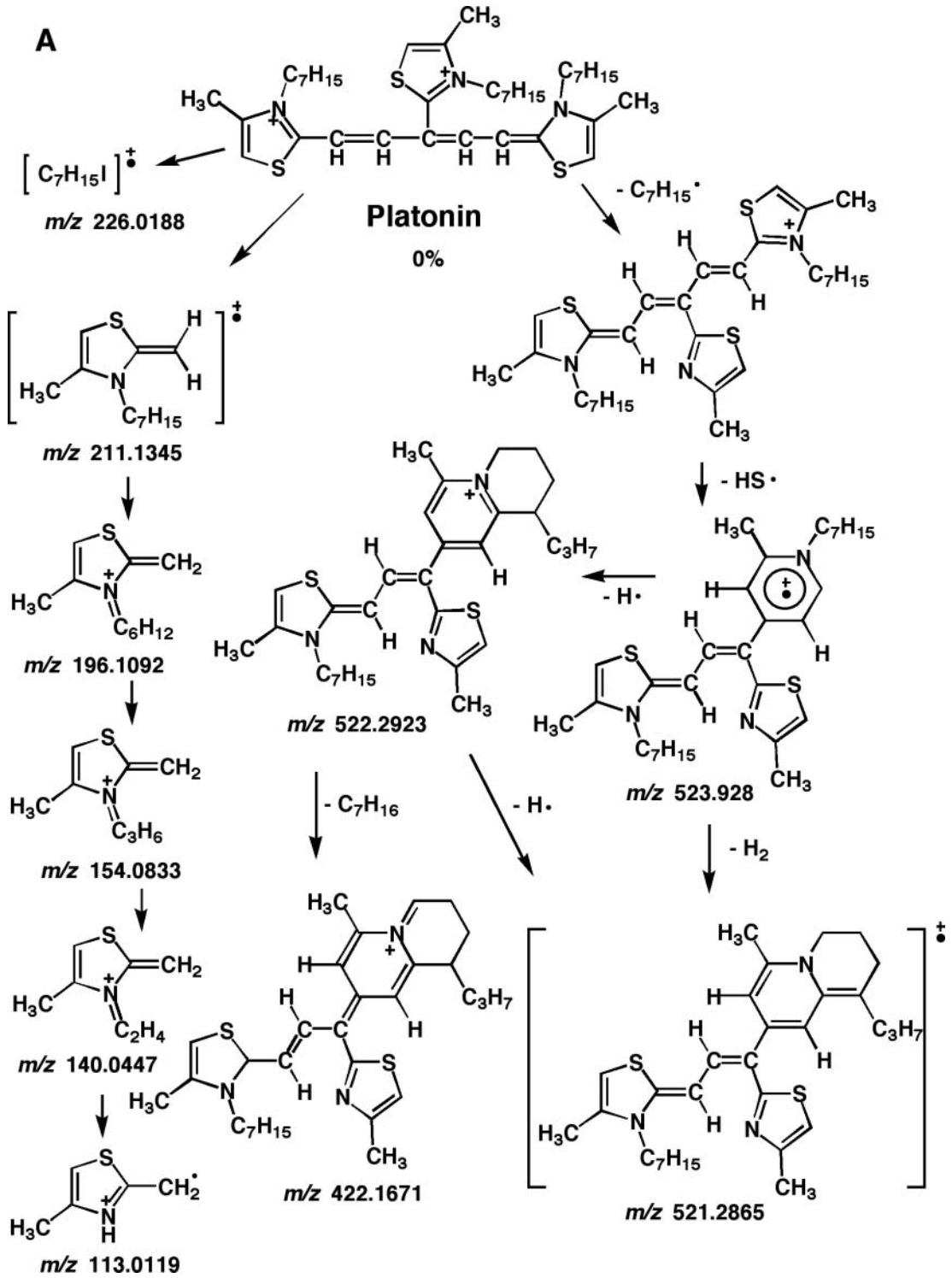

Figure 4. *continued*

HMT and HDT, especially the latter, greatly decreased oxygen uptake, accompanied by significant cell damage. The degree of decrease in oxygen uptake and increase of cytotoxicity were greater than that of platonin (37). HMT and HDT are thiazole derivatives, a class of compound that also includes sulfurol and 4-methyl-5-hydroxyethyl-thiazole, which are widely used as additives in the food industry and have also been utilized as antibiotics in the medical field. A

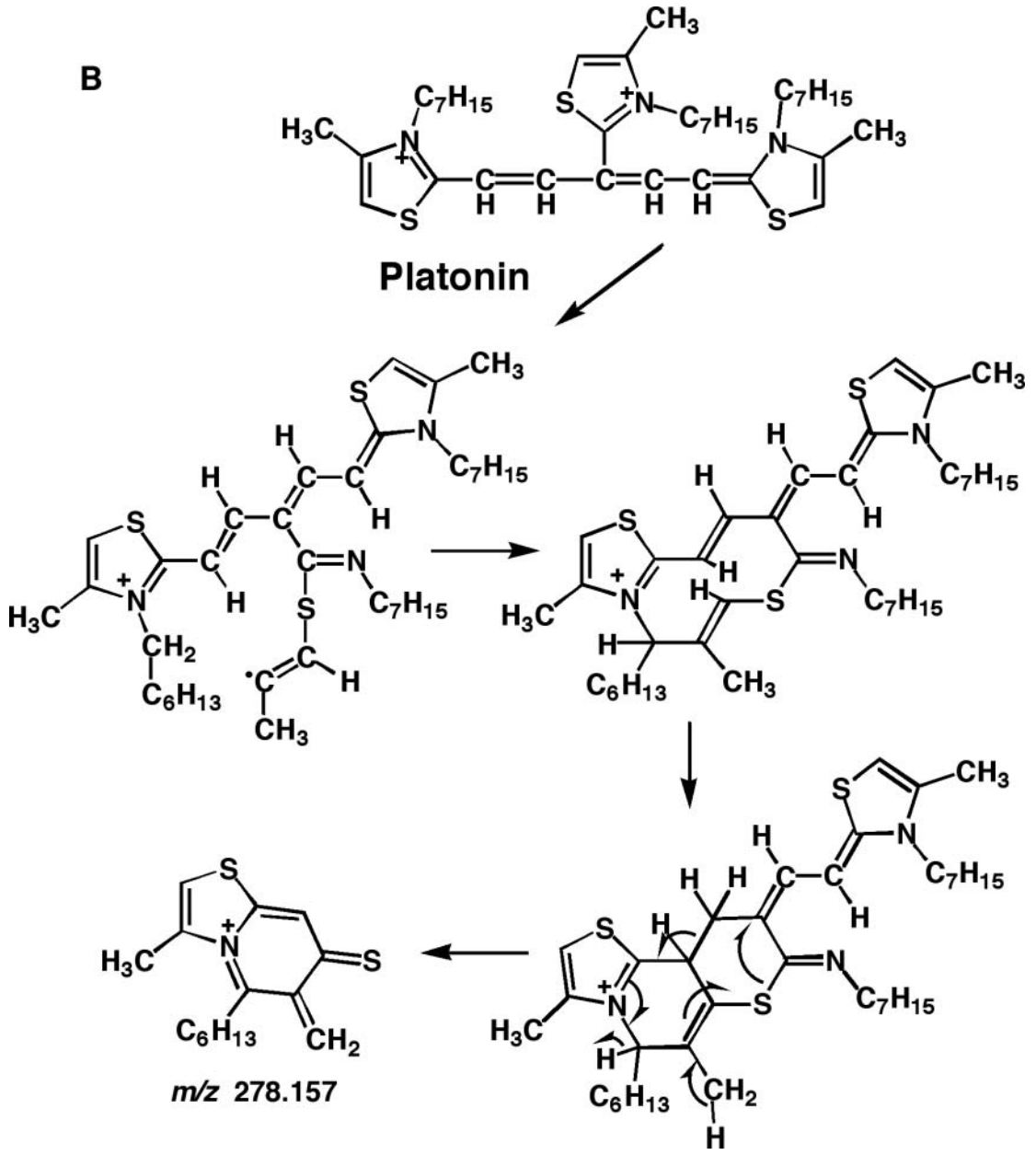

Figure 4. *A, B) Fragmentation of platonin. A (m/z: 113, 140, 154, 496, 211, 422, 521, 522 and 523) and B (m/z: 278).*

commercial compound, 2-(4-thiazolyl)benzimidazole (TBZ), is used as an antimicrobial in foods, textiles, papers and synthetic resins. Although HDT and HMT are cytotoxic, these compounds may possess antibacterial activity. Imidazole and benzimidazole derivatives are present in a large number of common therapeutic agents (38). A benzothiazole compound, 3-methyl-2-sulfonylmethylbenzothiazoline, has been synthesized (39) and possesses possible antibacterial activity. Thiazole derivatives having two thiazolium moieties in the molecule also exert strong bacteriostatic activity against gram-negative and -positive bacteria, and fungi (40). Substituted 2-(2-hydroxyphenyl) benzimidazoles have been previously evaluated *in vitro* for antibacterial activity against bacteria- associated periodontal disease, and are applicable as topical antibacterial agents (41). Moreover, 2-(4-aminophenyl)benzothiazole has been evaluated as an anticancer agent (42). Platonin, having tri-ring moieties in the molecule, shows antibacterial activity (1-3) and has been used for effective treatment of periodontal disease (3).

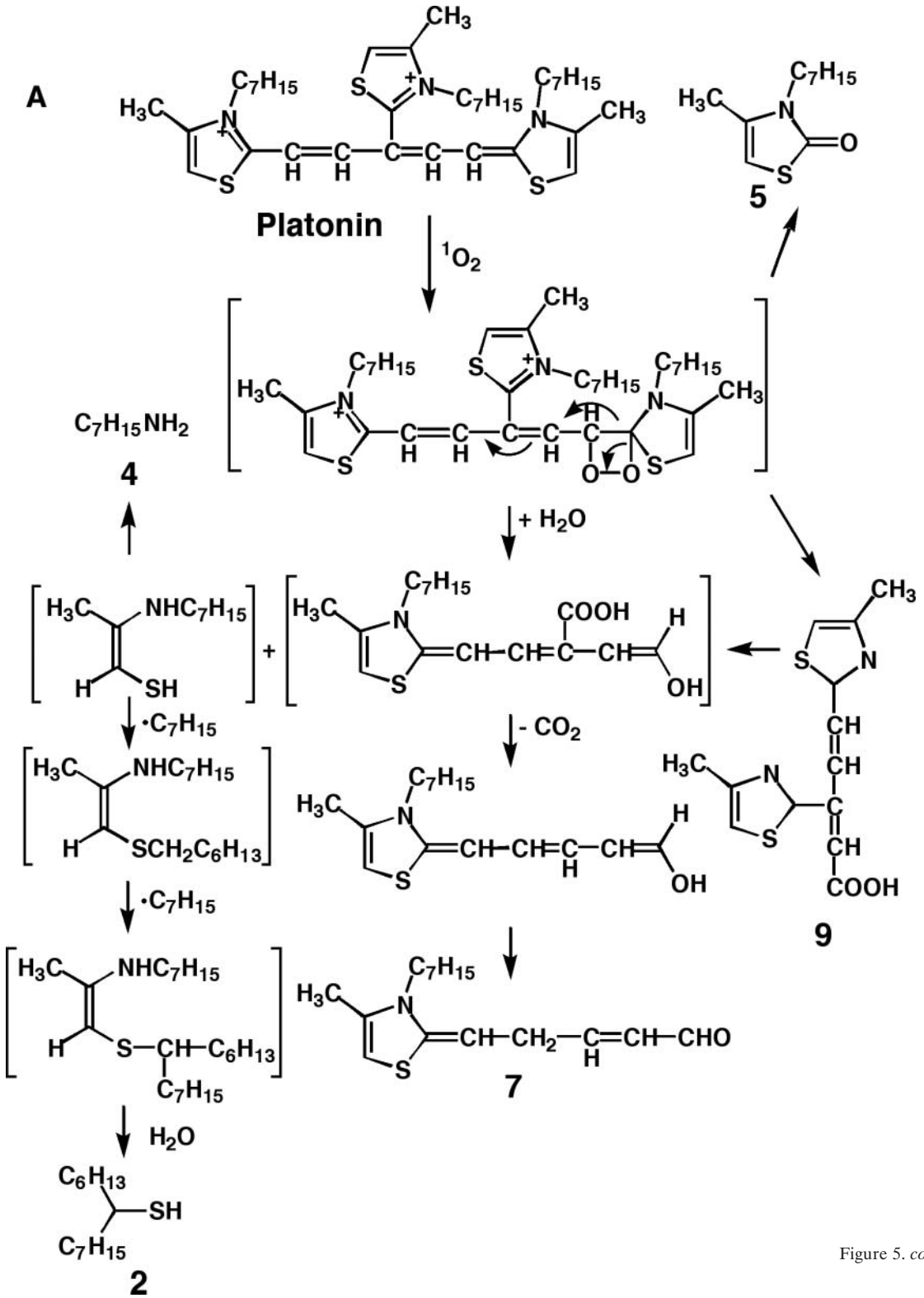

Figure 5. *continued*

Combination therapy. The pharmacological effects of platonin are much more enhanced when it is used in combination with other drugs. Platonin with steroids is known to be effective for the treatment of rheumatoid arthritis (43) and polyarteritis nodosa (44). The viability of rat hepatocytes treated with trapidil (triazolopyrimidine, an

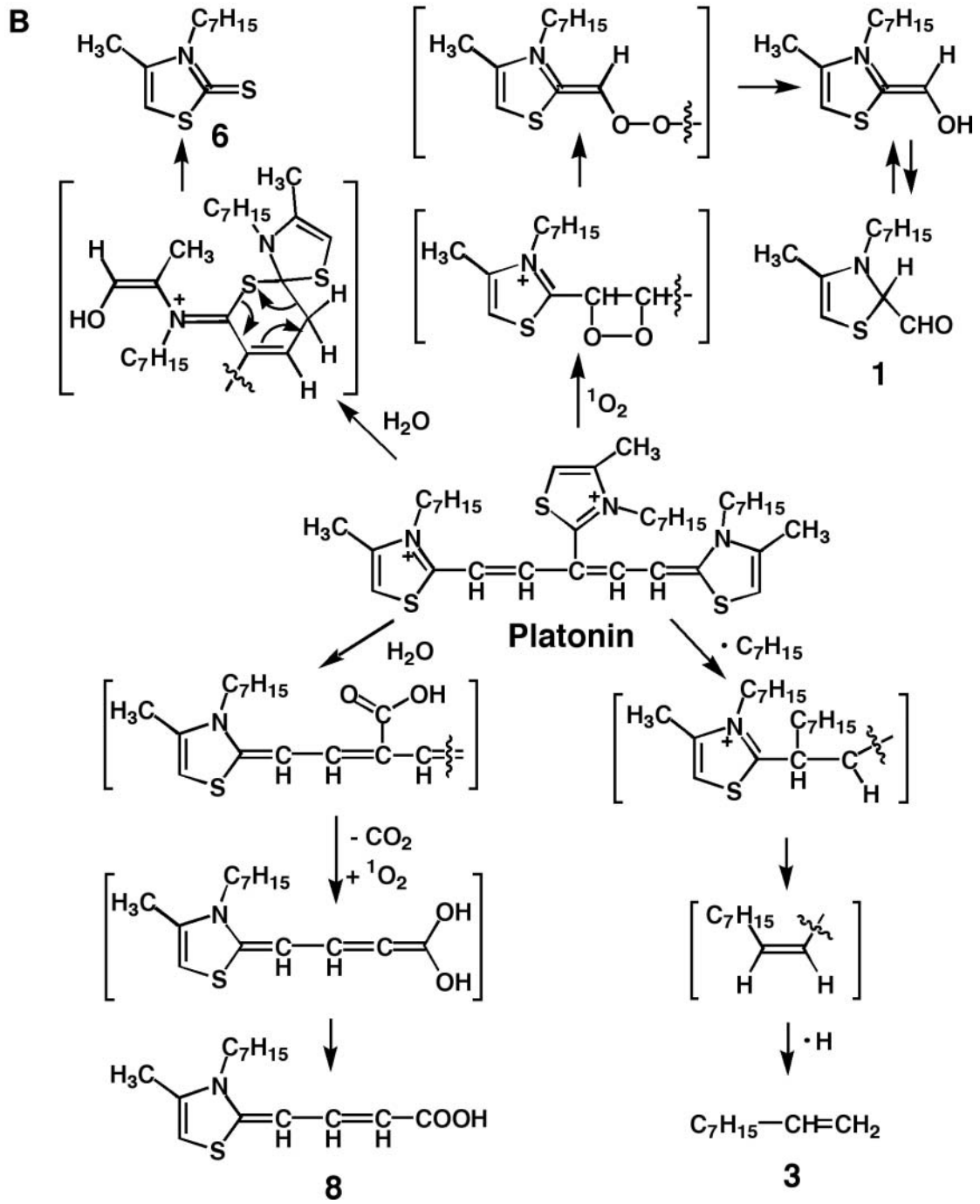

Figure 5. *A, B) A possible mechanism for the photodegradation of platonin. A (compounds:* ***2****,* ***4****,* ***5****,* ***7*** *and* ***9****) and B (compounds:* ***1****,* ***3****,* ***6*** *and* ***8****).*

inhibitor of phosphodiesterase and platelet-derived growth factor) and rifampicin (an anti-tuberculosis drug) in combination with platonin is slightly but significantly reduced in comparison to treatment with each drug alone. Although the mechanism of cytotoxicity is unknown, a beneficial effect of mixtures of platonin with trapidil or rifampicin has been recommended (45), but no clinical studies have been carried out. The effects of platonin with steroid hormones on bone wound healing have been reported, indicating that a combination of platonin with testosterone propionate promotes bone growth and mineralization (46).

Platonin was reported to be markedly effective against immunological suppression caused by administration of chemotherapeutic agents for cancer (47). Macrophage activity is well known to be synergistically enhanced by platonin administration with light irradiation (48) or without irradiation (49). Photodynamic cancer treatment using platonin with laser light irradiation in combination with ethanol is reportedly effective for cancer therapy in deep organs such as the liver (6). Thus, photodynamic treatment of cancer using platonin may be a promising avenue of investigation.

Conclusion

Platonin is a photosensitizing dye characterized by a typical absorption at 590 nm. VL-promoted photodegradation generates singlet oxygen molecules and yields degradation compounds, **1-6** (Figure 5), accompanied with heptyl and heptyl cation radicals. HMT and HDT, thiazole derivatives, show greater cytotoxicity than that of platonin, accompanied by a marked reduction of cellular oxygen uptake. Platonin is less cytotoxic and enhances cell energetics.

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