3,7-*Bis*(dialkylamino)phenothiazin-5-ium Derivatives: Biomedical Applications and Biological Activity

J.C.V.P. Moura[1,*] and N. Cordeiro[2]

[1]*Departamento de Química, Universidade do Minho, 4700-320 Braga, Portugal* and
[2]*Departamento de Química, Universidade da Madeira, 9000-081 Funchal, Portugal*

Abstract: The light-induced reactions of 3,7-*bis*(dialkylamino)phenothiazin-5-ium compounds with biological substrates are briefly discussed. Their biomedical applications, in particular those related with biological staining, interaction with proteins and antiviral, antibacterial and antitumour activity are reviewed.

**Key Words**: Methylene Blue Derivatives; Biomedical, Biological Activity.

INTRODUCTION

Dibenzo-1,4-thiazines, usually called phenothiazines, are a sub-group of the thiazine class, six-membered heterocycles containing one sulfur atom and a single nitrogen atom in which two benzene rings are fused to the heterocycle. Phenothiazine (**1**) was first reported by Bernthsen [1] and it was called thiodiphenylamine probably because it was obtained from sulfur and diphenylamine.

Phenothiazines represent an important class of drug substances which found application against several disorders. Their psychotherapeutic, chemotherapeutic, antihistaminic, antiemetic and antiseptic properties were recognised a long time ago. However, early interest in this group was more concerned with sulfur dye chemistry. Lauth´s violet (**2**), a purple dye, was prepared in 1876 by heating *p*-phenylenedi-amine with sulfur and treating the resulting hydrochloric acid solution with ferric chloride [2]. At the same time, in a similar reaction using *p*-aminodimethylaniline, methylene blue (MB) (**3**) was obtained [3,4].

MB (C.I. Basic Blue 9) is the most representative of 3,7-*bis*(dialkylamino)phenothiazin-5-ium compounds and its physico-chemical properties have found numerous applications. Examples include textiles (for dyeing cellulosic fibres and printing leather), antioxidants, antiseptics and photogalvanic cells based on redox systems. Moreover, its remarkable histochemical and photodynamic properties are of crucial importance for biology and medicine. MB has been used in histology for staining cellular components since the discovery of the nucleus of the malarial parasite by Romanowsky in 1891 [5] and it has more recently been recommended for photodynamic inactivation of blood viruses [6], this effect being known since 1965 [7].

Aspects concerning the synthesis [8-15] and the application [5,16-18] of this group of phenothiazines have been reviewed.

(**1**)

(**2**)

(**3**)
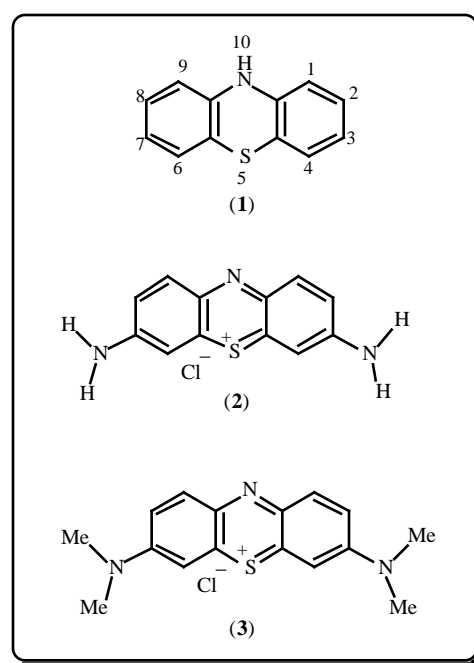
Examples of MB derivatives are shown below. Table **1** contains commercially available MB derivatives and Table **2** gives examples of symmetrical 3,7-*bis*(dialkylamino)pheno-thiazin-5-ium derivatives.

BIOMEDICAL APPLICATIONS

MB and analogues are employed in biology and medicine for many purposes. These include pH and redox indicators, staining of live and fixed tissues, enzyme reactions, anti-bacterial, anti-viral and anti-cancer agents.

(a) Biological Stains

Staining of live and fixed tissues is one of the major applications of synthethic dyes in biology. Among several

*Address correspondence to this author at the Departamento de Química, Universidade do Minho, 4700-320 Braga, Portugal;
Email: jmoura@quimica.uminho.pt

Table 1. Commercially Available MB Derivatives

(4)
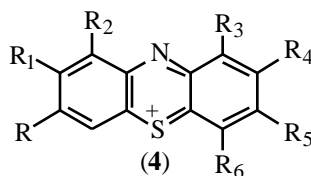
	R	R$_1$	R$_2$	R$_3$	R$_4$	R$_5$	R$_6$
(**4a**) Azure A	Me$_2$N	H	H	H	H	NH$_2$	H
(**4b**) Azure B	Me$_2$N	H	H	H	H	NHMe	H
(**4c**) Azure C	MeNH	H	H	H	H	NH$_2$	H
(**4d**) Methylene Green	Me$_2$N	H	H	H	H	NMe$_2$	NO$_2$
(**4e**) New Methylene Blue	EtNH	Me	H	H	Me	NHEt	H
(**4f**) Taylor´s Blue	Me$_2$N	H	Me	Me	H	NMe$_2$	H
(**4g**) Toluidine Blue O	Me$_2$N	H	H	H	Me	NH$_2$	H

Table 2. Symmetrical 3,7-*bis*(dialkylamino)phenothiazin-5-ium Derivatives

Compound		Reference
(**5**)	NR$_1$R$_2$ = ![piperazine]—N◯NMe	[19]
(**6**)	R$_1$ = Me; R$_2$ = C$_{18}$H$_{37}$	[20]
(**7**)	R$_1$ = R$_2$ = (CH$_2$CH$_2$Cl)$_2$	[19]
(**8**)	R$_1$ = R$_2$ = (CH$_2$CH$_2$OH)$_2$	[21]
(**9**)	R$_1$ = H; R$_2$ = Ph	[22]
(**10**)	R$_1$ = Me; R$_2$ = Ph	[23]
(**11**)	R$_1$ = H; R$_2$ = ![aryl]—◯—R R=Me, OMe, Oet, Cl, I	[23]
(**12**)	R$_1$ = R$_2$ = CH$_2$Ph	[23]
(**13**)	R$_1$ = H; R$_2$ = CH$_2$Ph	[23]
(**14**)	R$_1$ = Me; R$_2$ = (CH$_2$)$_3$CO$_2$H	[24]

different methods, staining with mixtures of acid and basic dyes is a widely employed technique because cell constituents carry both cationic and anionic charges. Malarie parasite was seen for the first time in 1891 by Romanowsky´s invention of MB eosinate, a mixture of MB, a basic dye, with a slight excess of eosine, an acid dye (tetrabromofluorescein disodium salt) in water [5]. Several modifications of

this method have been proposed in order to produce different colour effects in a blood smear: purple (chromatin of leukocytes), red (nucleus of parasitic protozoa), blue (monocytes) and pink (eosinophilic granules).

Azure A (**4a**), B (**4b**) and C (**4c**), the demethylated analogues of MB, prepared by treating MB in alkaline

solution, have been used as a mixture, known as Poly-chrome MB, for staining purposes [25].

MB derivatives are important dyes for the induction of metachromasia, a phenomenon associated with the ability of dyes to colour different tissue constituents in different colours. Taylor and Jeffree [12] studied the metachromatic properties of 1,9-dimethylmethylene blue (Taylor´s Blue) (**4f**), compared with MB, Azure B (**4b**) and Toluidine Blue O (TBO) (**4g**), a well-known metachromatic dye. Results obtained with 1,9-dimethylmethylene blue (**4f**) gave a consistently more intense metachromasia than any of the other three dyes tested.

TBO (**4g**) is one of the most popular dyes for staining microorganisms and provides a method for the diagnosis of several diseases [26] and MB, Azures A (**4a**) and B (**4b**) have been used as vital dyes for the coloration of unfixed tissues in their living state [5].

(b) Interaction with Proteins

It has been known for a long time, that certain compounds are photoactive and can cause damage to biological substrates such as bacteria, carcinomas, viruses and proteins. These compounds, known as photosensitizers can be light activated into excited states and, after returning to the ground state, can generate DNA modifications.

These modifications have mutagenic [27] and potentially carcinogenic consequences [28]. Several authors confirmed the preferencial destruction of guanine bases on cleavage of DNA [29-32].

There is evidence that MB binds intercalatively to DNA [33] preferentially at guanine residues [32,34-36]. Gutter, Speck and Rosenkranz [37] demonstrated the ability of MB to photooxidize the guanine moiety of intracellular DNA of living human cells by immuno-fluorescence using an antibody with a specificity for the unpaired cytosine residue of DNA.

Recently, a single-cell electrophoresis (comet assay) has been used to evaluate DNA damage and repair in the human myeloid leukemia cell line K562 after photodynamic therapy [38]. According to the authors, this technique can be applied as an effective screening assay for DNA damage induced by a range of laser therapies.

MB intercalates strongly to DNA in chromatin [39]. Lalwani, Maiti and Mukherji [40-43] studied the formation of DNA-protein crosslinks by the action of visible light in the presence of MB. The results showed that intercalation of MB with DNA of chromatin is essential for crosslink formation, resulting on stabilization of the compact structure of chromatin.

The effect of MB sensitized photooxidation of collagen fibrils has been studied [44]. The results showed that visible light in the presence of MB led to an increase in the termal stability of the collagen.

Photoactive dyes are also useful as labeling agents in immunodiagnostics. It has been synthesized several MB derivatives having succinimido and maleimido residues which can be coupled with amino or sulfhydryl residues of proteins, respectively [45]. The authors suggested that these compounds should be useful for photodynamic tumor therapy and immunodiagnostics where it is desired to attach a red-absorbing photoactive dye to antibody protein.

A method using a mixture of ethyl formate and propylene glycol monomethyl ether for stabilization of phenothiazine dyes for clinical diagnosis has been proposed [46].

Fibrinogen is one of the most abundant proteins in plasma and also one of the most important coagulation factors. The lowering of coagulability of human plasma may lead to prevention of thrombosis. It has been shown that MB phototreatment of plasma proteins reduces the coagulability of blood plasma [47,48]. This phenomenon is associated with the photooxidation of the histidine residue located at position 16 in the B -chain of the fibrinogen molecule [49].

Chepurov and Lazarenko on studying the effect of TBO (**4g**), Azure A (**4a**) and 1,9-dimethylmethylene blue (**4f**) on blood coagulation, after I.V. injection into rabbits, observed hypofibrinogenemia and thrombocytopenia of the blood [50].

Other phenothiazine dyes have been suggested for biochemical applications. These include the use of TBO (**4g**), a strong photoactive agent [51], for simultaneous determination of blood thrombocytes and leukocytes [52], measurement of genomic DNA extracted from peripheral blood leukocytes [53] and as cell nuclei staining agent [54].

1,9-dimethylmethylene blue (**4f**) can be used to distinguish between DNA and tRNA through differences in induced circular dichroism [55].

MB and MB derivatives have the capacity to photooxidize amino acids [56] and this reactions can occur by both Type I (proteins react directly with the triplet state of the sensitizer) or Type II (singlet oxygen reaction) pathways. These reactions occur with isolated amino acids and also when they are incorporated into enzymes. Such changes occuring in the protein molecules can interfere with enzyme activity causing irreversible chemical changes leading to inactivity.

Studies on the photoinactivation of enzymes by MB have been performed. Examples include alcohol dehydrogenase [57,58], lysozyme [59-61], tyrosinase [62], asparaginase [63], transferase [64,65], formyltetrahydrofolate synthetase [66], dehydrogenase [67,68], papain [69], creatine Kinase [70], ribonuclease [71], arginase [72] and protease [73].

Müeller, Boiteux and Cunningham [27] confirmed that endonucleases are responsible for enzymic recognition of DNA modifications induced by singlet oxygen and photosensitizers.

Experiments in rats with MB and UV light (366 nm) showed that MB enhanced photorelaxation in aorta, pulmonary artery and corpus cavernosum [74]. According to

the authors, UV-generated free radicals convert the phenothiazine moiety of MB to a phenothiazine radical which activates guanylate cyclase and thus enhances smooth muscle relaxation.

BIOLOGICAL ACTIVITY

(a) Antiviral Activity

One of today's problems in the medical field, is the risk of infection from pathogenic contaminants in handling or being transfused with blood or blood derivates. Because of the critical need for transfusible red blood cells and platelets, it is of great importance to develop methods that can be readily used to decontaminate cellular blood components and whole blood without substantially or irreversibly altering or harming them.

Virucidal methods, including heat, filtration, solvent-detergent, steam sterilization and gamma irradiation, have been used for effective decontamination of whole blood, red cells and platelets. Unfortunatelly, such methods are generally ineffective or too harsh to be routinely used. A more recent approach to viral inactivation is the treatment of blood with a photochemical agent and light. Common decontamination treatments include the use of MB and analogues, which, in the presence of oxygen and upon exposure to light that include wavelenghts absorbed by the photosensitizer, inactivate viruses [6,75-78]. The efficiency of MB on the photoinactivation of human immunodeficiency virus (HIV) has been studied [79,80].

Bachmann, Knüver-Hopf, Lambrecht and Mohr [81] examined the mechanism of photodynamic inactivation of HIV-1 by MB and suggested that it acts at different target sites: the envelope and core proteins, the inner core RNA structures and reverse transcriptase.

Recently, it has been proposed a method for the inactivation of HIV in blood products by applying MB as photosensitizer and visible light [82].

MB/light treatment of blood or blood components inactivates hepatitis C virus [83], herpes simplex virus [84,85], vesicular stomatitis virus [86], Junin virus [87], herpes virus hominis [88] and tobacco mosaic virus [89].

MB is also effective on irreversible inactivation of certain bacteriophages by inducing letal lesions in RNA bacteriophages [90]. Examples include the bacteriophage ØX 174 [91], bacteriophage PM2 [92], M13 bacteriophage [93,94] and Qβ bacteriophage [95,96].

Other MB derivatives have been recommended for the decontamination of blood and cellular components. They include Azure A (**4a**), B (**4b**), and C (**4c**) [97], and 1,9-dimethylmethylene blue (**4f**) [98-101]. TBO proved to be the most efficient for the photoinactivation of bacteriophage Ø6 [102], phage PM2 [103], murine myocarditis virus [104], HIV-1 [105] and adenoviruses [106].

(b) Antibacterial Activity

Escherichia coli represents the most studied bacterial species in relation to photochemical inactivation by phenothiazine dyes, particularly, MB [107-109]. MB is a weak mutagen and it has been shown that its combination with light induces minor changes in *E. coli* ribosomes [110] and stable lesions in DNA which could be repaired by various cellular repair systems [111].

The light-induced mutagenic effect of MB on *E. coli* was studied by Tudek, Laval and Boiteux [112] and it was suggested that 8-oxo-7,8-dihydroguanine is the major promutagenic lesion in DNA. Capella and Menezes [113] showed that the resistance of *E. coli* cells to the photodynamic action of MB is increased by the addition of glucose to the media in which they are grown. According to the authors, this effect may be due to lower retention of the dye by cells grown in the presence of glucose, leading to the diminution in DNA damage.

Hyperthermia treatment is shown to act synergistically with MB, from the end point of lethality in gram negative *E. coli* cells [114]. The role of temperature appeared to be to facilitate the incorporation of the dye, which enables the later to intercalate into the DNA.

The same synergistic effect was also observed between electrolysis and MB photodynamic action when weak electric currents (1.0 mA) were applied to *E. coli* cells [115].

The phototoxicity effect of MB derivatives such as (**15**) and analogues towards *E. coli* and other microorganisms (coliforms and enterococci) can be used for disinfecting water [116] or aqueous domestic effluents [117].

Other bacterial species are photoinactivated by MB. They include *Helicobacter pylori* [118] and *Helicobacter mustelae* [119], *Streptococcus mutans* [120], *Proteus mirabilis* [121], *Propionibacterium acnes* [122], *Mycoplasma* [123], *Salmonella typhimurium* [124,125], *Bacillus mesentericus* [126] and *Tetrahymena pyriformis* [127]. Other microorganisms


(**15**)
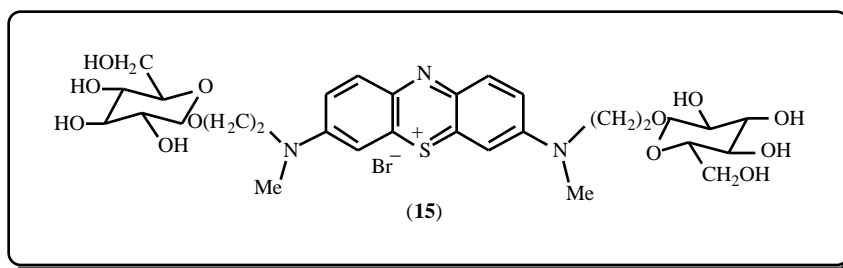
such as *Paramecium* [128,129] and *Pieris brassicae* larvae [130] are also photoinactivated by MB.

TBO (**4g**) is another dye widely studied for the photodynamic inactivation of microorganisms. Examples include *Sarcina lutea* [131], *Micrococcus roseus* [132], pararickettsia *Clamida psittaci* [133], Clamydia ornithosis [134], *Staphylococcus aureus* [135,136] and *Escherichia coli* [109,137-139].

Recent studies have shown that cariogenic bacteria such as *Streptococcus mutans* can be killed when exposed to low power laser light in the presence of TBO, suggesting that lethal photosensitization may be useful as a means of eliminating plaque bacteria from a carious lesion prior to its restoration [140-142].

It has tested the photodynamic antibacterial properties of a closely related series of commercially available phenothiazine dyes against a range of pathogenic strains of Gram-positive and Gram-negative organisms [143]. In several cases, illumination resulted in considerable decreases in the minimum lethal concentration required, giving up to 100-fold increases in bactericidal activity.

New methylene blue (**16**) was photodynamically active against epidemic strains of methicillin-resistant *Staphylococcus aureus* [144].

(**16**)
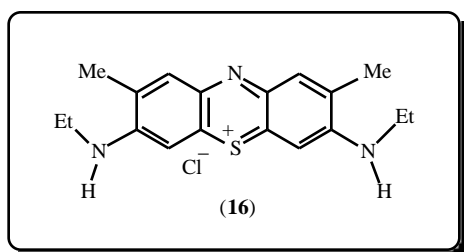
The photodynamic effect of MB analogues on several eukaryotic organisms has been investigated. TBO is able to sensitise *Candida albicans* and other *Candida* species to killing by light from HeNe laser light [145]. Ito studied the TBO-photodynamic action in yeast cells and confirmed the membrane damage as a determining step for the inactivation of yeast cells [146,147].

Photodynamic treatment of yeast with TBO results in loss of cell viability. The photosensitizer enters the cell during the first minutes of illumination, whereafter intracellular enzymes are inactivated [148-150].

Lazarova and Palazov proposed a method for the prevention of TBO-photodynamic yeast cell damage by substances possessing radioprotective activity such as 2-aminoethylisothiouronium bromide [151]. According to the authors, these results indicate that prevention of photodynamic yeast cell damage by radioprotectors could find application as a method for determination of the radioprotective effects of biotechnologically obtained substances during their production and purification.

Fungi have also been subjected to TBO photodynamic treatment. The photoinactivations of *Neurospora crassa* conidia [152], *Ustilago violacea* [153] and *Saccharomyces cerevisiae* [154,155] have been reported.

(c) Antitumour Activity

Recently, Photodynamic Therapy (PDT) has attracted pronounced medical interest for the treatment of tumours. MB and MB analogues have received attention because of their selective uptake by mitochondria of carcinoma cells [156].

The mechanism of cytophototoxicity has been proposed to be primarily due to the dye sensitized photogeneration of highly toxic singlet oxygen ($^1O_2$) in the mitochondria [157]. It has been examined the induction of apoptosis by a positively charged MB derivative DO15 (**17**) [158]. Photodynamic treatment yielded mitochondrial photodamage while membrane and lysosomal integrity were maintained. According to the authors, this is the first report of a thiazine photosensitizer inducing apoptosis and is consistent with recent proposals suggesting that release of mitochondrial components may play an important role in the mechanism of cell death.

In order to investigate the MB-PDT of cancer, studies have been carried out with cancer cells. It has been examined the biodistribution of a MB derivative (**17**) in tumour and normal tissue of rats using Wistar rats bearing fibrosarcoma [159]. Four hours after injection, selective uptake of the dye by the tumour was observed. The dye was localized on the walls of all the vessels and extensively in the area of neoplastic cellular and tumorigenic fibrous components in the tumour tissue.

(**17**)
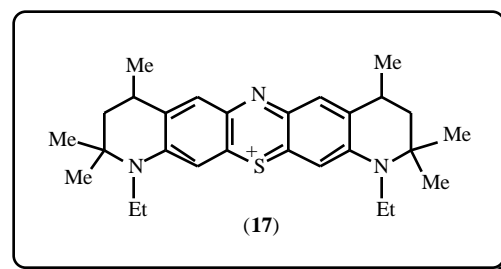
Tumour localisation of bladder carcinomas using MB or TBO can be achieved by staining techniques [160,161] including chromocystoscopy. In this technique, *in vivo* intravesical application of MB or TBO to malignant or premalignant bladder urothelium, results in diagnostically-useful differential surface staining [162,163].

MB has also been recommended for the photodynamic treatment of nonmalignant diseases such as psoriasis [164].

CONCLUSION

3,7-*Bis*(dialkylamino)phenothiazin-5-ium compounds have been used for a long time and have found many applications

other than just as colorants. Today, there is still a major interest in them, particularly in the biomedical area, and developments are likely to continue for a long time to come.

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REFERENCES

[1]   Bernthsen, A. (1883) *Ber. Deut. Chem. Ges.,* **16**, 2896-2904.

[2]   Lauth, C. (1876) *Ber. Deut. Chem. Ges.,* **9**, 1035-1036.

[3]   Caro, H. (1877) *Engl. Pat.* 3751 (9 Oct 1877).

[4]   Bernthsen, A. (1885) *Liebigs Ann. Chem.,* **230**, 73-137.

[5]   Gurr, E.; Anand, N.; Unni, M.K. and Ayyangar, N.R. (1974) in *The Chemistry of Synthetic Dyes,* vol. III, (Venkataraman, K., Ed.) Academic Press, pp. 278-351.

[6]   Chapman, J. and Mathias, J.M. (1998) *Patent Cooperation Treaty (PCT) Int. Appl.* W.O. 98 28607.

[7]   Wallis, C. and Melnick, J.L. (1965) *Photochem. Photobiol.,* **4**, 159-170.

[8]   Kehrmann, F. and Speitel, R. (1916) *Ber. Deut. Chem. Ges.,* **49**, 53-54.

[9]   Meyer, V. and Jacobsen, P. (1920) in *Lehrbuch der Organischen Chemie,* vol. II, part 3, Veit and Co., p. 1490.

[10]   David, F. and Blangey, L. (1949) in *Fundamental Processes of Dye Chemistry,* Interscience, New York, 311-314.

[11]   Bodea, C. and Silberg, I. (1968) in *Advances in Heterocyclic Chemistry,* vol. IX, (Katritzky, A.R. and Boulton, A.J., Ed.), Academic Press, New York, pp. 321-460.

[12]   Taylor, K.B. and Jeffree, G.M. (1969) *Histochem. J.,* **1**, 199-204.

[13]   Coffey, S. (1978) in *Rodd´s Chemistry of Carbon Compounds,* vol. IV, part H, (Coffey, S., Ed.) Elsevier Scientific Publishing Co., Amsterdam, pp. 516-535.

[14]   Albery, W.J.; Bartlett, P.N. Lithgow, A.M.; Riefköhl, J.L.; Rodriguez, L.A.; Romero, L. and Souto, F.A. (1985) *J. Org. Chem.,* **50**, 596-603.

[15]   Strekowski, L.; Hou, D.F.; Wydra, R.L. and Schinazi, R.F. (1993) *J. Heterocyclic Chem.,* **30**, 1693-1695.

[16]   Tuite, E.M. and Kelly, J.M. (1993) *J. Photochem. Photo-biol. B: Biol.,* **21**, 103-124.

[17]   Hallas, G. and Towns A.D. (1995) in *Supplements to the 2nd Edition of Rodd´s Chemistry of Carbon Compounds,* vol. IV I/J, (Ansell, M.F., Ed.) Elsevier Science B.V., Amsterdam, pp. 193-221.

[18]   Schenker, E. and Herbst, H. (1963) *Progr. Drug Res.,* **5**, 269-627.

[19]   Hoffmann, E. and Werner, E. (1969) *Ger. Pat.* 1906527.

[20]   Valenty, S.J. (1979) *J. Colloid. Interface Sci.,* **68**, 486-491.

[21]   Creed, D.; Burton, W. and Fawcett, N.C. (1983) *J. Chem. Soc. Chem. Commun.,* 1521-1523.

[22]   Andreani, F.; Bizzarri, P.C.; Casa, C.D.; Fiorini, M. and Salatelli, E. (1991) *J. Heterocyclic Chem.,* **28**, 295-299.

[23]   Wainwright, M.; Grice, N.J. and Pye, L.E.C. (1999) *Dyes Pigm.,* **42**, 45-51.

[24]   Moura, J.C.V.P.; Oliveira-Campos, A.M.F. and Griffiths, J. (1997) *Phosphorus, Sulfur Silicon,* **120 & 121**, 459-460.

[25]   Gurr, E. (1964) *Nature (London),* **202**, 1022-1023.

[26]   Moulder, J.W.; Lewert, R.M. and Rippon, J.W. (1968) in *Textbook of Microbiology,* 19th Ed., Saunders, Philadelphia, pp. 18 and 47.

[27]   Müeller, E.; Boiteux, S. and Cunningham, R.P. (1990) *Nucleic Acids Res.,* **18**, 5969-5973.

[28]   Buchko, G.W.; Cadet, J.; Morin, B. and Weinfeld, M. (1995) *Nucleic Acids Res.,* **23**, 3954-3961 (references cited therein).

[29]   Simon, M.I. and Van Vunakis, H. (1962) *J. Mol. Biol.,* **4**, 488-499.

[30]   Friedman, T. and Brown, D.M. (1978) *Nucleic Acids Res.,* **5**, 615-622.

[31]   OhUigin, C.; McConell, D.J.; Kelly, J.M. and van der Putten, W.J.M. (1987) *Nucleic Acids Res.,* **15**, 7411-7427.

[32]   Blau, W.; Croke, D.T.; Kelly, J.M.; McConnell, D.J.; OhUigin, C. and Van der Putten, W.J.M. (1987) *J. Chem. Soc., Chem. Commun.,* (10), 751-752.

[33]   Bradley, D.F.; Stellwagen, N.C.; O´Konski, C.T. and Paulson, C.M. (1972) *Biopolymers,* **11**, 645-652.

[34]   Müller, W. and Crothers, D.M. (1975) *Eur. J. Biochem.,* **54**, 267-277.

[35]   Piette, J.; Decuyper, J.; Merville-Louis, M.P. and Van de Vorst, A. (1986) *Biochimie,* **68**, 835-842.

[36]   Feldberg, R.S.; Brown, C.; Carew, J.A. and Lucas, J.L. (1983) *Photochem. Photobiol.,* **37**, 521-524.

[37]   Gutter, B.; Nishioka, Y.; Speck, W.T.; Rosenkranz, H.S.; Lubit, B. and Erlanger, B.F. (1976) *Exp. Cell. Res.,* **102**, 413-416.

[38]   Mcnair, F.I.; Marples, B.; West, C.M.L. and Moore, J.V. (1997) *Br. J. Cancer,* **75**, 1721-1729.

[39]   Wang, J.; Hogan, M. and Austin, R.H. (1982) *Proc. Natl. Acad. Sci. USA,* **79**, 5896-5900.

[40]   Lalwani, R.; Maiti, S. and Mukherji, S. (1990) *J. Photochem. Photobiol. B,* **7**, 57-73.

[41]   Lalwani, R.; Maiti, S. and Mukherji, S. (1991) *Indian J. Phys.*, **65B** (6), 629-634.

[42]   Lalwani, R.; Maiti, S. and Mukherji, S. (1995) *J. Photochem. Photobiol. B*, **27**, 117-122.

[43]   Maiti, S.; Lalwani, R. and Mukherji, S. (1991) *Indian J. Phys.*, **65B** (1), 111-116.

[44]   Ramshaw, J.A.M.; Stephens, L.J. and Tulloch, P.A. (1994) *Biochim. Biophys. Acta*, **1206** (2), 225-230.

[45]   Motsenbocker, M.; Masuya, H.; Shimazu, H.; Miyawaki, T.; Ichimori, Y. and Sugawara, T. (1993) *Photochem. Photobiol.*, **58**, 648-652.

[46]   Yoneda, J. and Eguchi, T. (1995) *Jap. Pat.* 07 234 221.

[47]   Zeiler, T.; Riess, H.; Wittmann, G. and Huhn, D. (1994) *Transfusion (Bethesda, MD)*, **34**, 685-689.

[48]   Yoshimoto, Y.; Saito, Y. and Inada, Y. (1987) *Photochem. Photobiol.*, **45**, 675-676.

[49]   Shimizu, A.; Saito, Y.; Matsushima, A. and Inada, Y. (1983) *J. Biol. Chem.*, **258**, 7915-7917.

[50]   Chepurov, A.K. and Lazarenko, G.I. (1977) *Byull. Eksp. Biol. Med.*, **84**, 279-282. [*Chem. Abstr.*, **87**, 194034d (1977)].

[51]   Hazen, M.J. and Molero, M.L. (1994) *Ver. Toxicol.*, **11**, 82-86.

[52]   Muszbek, L.; Roza, A.; Debrecen, H.U.; Harsanyi, I.; Zajka, G. and Budapest, H.U. (1985) *Ger. Pat. DE* 3432351 A1.

[53]   Passmore, L.J. and Killeen, A.A. (1996) *Mol. Diagn.*, **1**, 329-334.

[54]   Fischer, W. and Renate, L. (1982) *Ger. Pat. DE* 3112442 A1.

[55]   Pal, M.K. and Yadav, R.C. (1990) *Indian J. Chem.*, **29A**, 723-728.

[56]   Knowles, A. and Gurnani, S. (1972) *Photochem. Photobiol.*, **16**, 95-108.

[57]   Nilsson, R. and Kearns, D. R. (1973) *Photochem. Photobiol.*, **17**, 65-68.

[58]   Julliard, M.; Le Petit, J. and Ritz, P. (1986) *Biotechnol. Bioeng.*, **28**, 1774-1779.

[59]   Schmidt, H.; Al-Ibrahim, A.; Dietzel, U. and Bieker, L. (1981) *Photochem. Photobiol.*, **33**, 127-130.

[60]   Silva, E. and Gaule, J. (1977) *Radiat. Environ. Biophys.*, **14**, 303-310.

[61]   Churakova, N.I.; Kravchenko, N.A.; Serebryakov, E.P.; Lavrov, I.A. and Kaverzneva, E.D. (1973) *Photochem. Photobiol.*, **18**, 201-204.

[62]   Pfiffner, E. and Lerch, K. (1981) *Biochemistry*, **20**, 6029-6035.

[63]   Makino, H. and Inada, Y. (1973) *Biochim. Biophys. Acta*, **295**, 543-548.

[64]   Nishiyama, M. (1972) *Shikoku Igaku Zasshi*, **28**, 299-307. [*Chem. Abstr.*, **77**, 98303p (1972)].

[65]   Gutensohn, W. and Jahn, H. (1980) *Adv. Exp. Med. Biol.*, 122B, 117-122.

[66]   Mackenzie, R.E.; Strauss, L.A. and Rabinowitz, J.C. (1972) *Arch. Biochem. Biophys.*, **150**, 421-427.

[67]   Saito, M. (1976) *Annu. Rep. Res. React. Inst., Kyoto Univ.*, **9**, 55-61. [*Chem. Abstr.*, **86**, 116627q (1977)].

[68]   Rasched, I.; Bohn, A.; David, M. and Sund, H. (1975) *Biochem. Soc. Trans.*, **3**, 926-927.

[69]   Ohara, A.; Fujimoto, S.; Kanazawa, H. and Nakagawa, T. (1975) *Chem. Pharm. Bull.*, **23**, 967-970.

[70]   Chetverikova, E.P. (1973) *Biofizika*, **18**, 365-369. [*Chem. Abstr.*, **79**, 50219e (1973)].

[71]   Hashimoto, J.; Takashashi, K. and Uchida, T. (1973) *J. Biochem.*, **73**, 13-22.

[72]   Ozan, S. and Gulen, S. (1991) *Doga Turk. Biyol. Derg.*, **15** (3), 222-229. [*Chem. Abstr.*, **116**, 169059a (1992)].

[73]   Kaneda, M.; Tomita, Y. and Tominaga, N. (1987) *Experientia*, **43**, 318-319.

[74]   Chen, X. and Gillis, C.N. (1993) *Biochem. Biophys. Res. Commun.*, **190**, 559-563.

[75]   Dolana, G.H. (1986) *E.P.* 196515 A1.

[76]   Chapman, J.R.; Stark, P.R.H.; Reed, M.A.; Larson, D.N. and Cuffaro, D.F. (1998) *Patent Cooperation Treaty (PCT) Int. Appl.* W.O. 98 22150.

[77]   Wagner, S.J. (1996) *U.S. Pat.* 5 545 516.

[78]   Struckmeier, A.K. and Mohr, H. (1997) *Beitr. Infusionsther. Transfusionsmed.*, **34**, 43-47.

[79]   Lambrecht, B.; Norley, S.G.; Stephen, G.; Kurt, R. and Mohr, H. (1994) *Biologicals*, **22**, 227-231.

[80]   Schinazi, R.F. (1990) *Patent Cooperation Treaty (PCT) Int. Appl.* W.O. 90 13296.

[81]   Bachmann, B.; Knüver-Hopf, J.; Lambrecht, B. and Mohr, H. (1995) *J. Med. Virol.*, **47**, 172-178.

[82]   Brockmeyer, N.H.; Hoffmann, K.; Altmeyer, P.; Alamouti, D.; Saalmann, G. and Saalmann, P. (2000) *Ger. Offen. D.E.* 19914850.

[83]   Breitkreutz, K.M. and Mohr, H. (1998) *J. Med. Virol.*, **56**, 239-245.

[84]   Breitkreutz, K.M. and Mohr, H. (1997) *Beitr. Infusionsther. Transfusionsmed.*, **34**, 37-42.

[85]   Swartz, M.R.; Schnipper, L.E.; Lewin, A.A. and Crumpacker, C.S. (1979) *Proc. Soc. Exp. Biol. Med.*, **161** (2), 204-209.

[86]   Abe, H. and Wagner, S.J. (1995) *Photochem. Photobiol.*, **61**, 402-409.

[87]   Cobo, M.F.; Pasian, E.L. and Segovia, Z.M.M. (1986) *Med. Microbiol. Immunol.*, **175**, 67-69.

[88]   Chang, T.W. and Weinstein, L. (1975) *Proc. Soc. Exp. Biol. Med.*, **148**, 291-293.

[89] Yoshizaki, T. (1986) *Hokkaido Kyoiku Daigaku Kiyo, Dai-2-Bu, B*, **36**, 7-15. [*Chem. Abstr.*, **105**, 187519c (1986)].

[90] Schneider, J.E.; Phillips, J.R.; Pye, Q.; Maidt, M.L.; Price, S. and Floyd, R.A. (1993) *Arch. Biochem. Biophys.*, **301**, 91-97.

[91] Houba-Herin, N.; Bacq, C.M.C.; Piette, J. and Van de Vorst, A.V. (1982) *Photochem. Photobiol.*, **36**, 297-306.

[92] Gonikberg, E.M. and Kuznetsova, N.V. (2001) *Biofizika*, **43**(3), 486-493.

[93] Abe, H.; Ikebuchi, K.; Wagner, S.J.; Kuwabara, M.; Kamo, N. and Sekiguchi, S. (1997) *Photochem. Photobiol.*, **66**, 204-208.

[94] Abe, H.; Wagner, S.J.; Kuwabara, M.; Kamo, N.; Ikebuchi, K. and Sekiguchi, S. (1997) *Photochem. Photobiol.*, **65**, 873-876.

[95] Jockusch, S.; Lee, D.; Turro, N.J. and Leonard, E.F. (1996) *Proc. Natl. Acad. Sci. USA*, **93**, 7446-7451.

[96] Lee, D.; Foux, M. and Leonard, E.F. (1997) *Photochem. Photobiol.*, **65**, 161-165.

[97] Mohr, H.; Bachmann, B.; Struckmeier, A.K. and Lambrecht, B. (1997) *Photochem. Photobiol.*, **65**, 441-445.

[98] Wagner, S.J. and Cincotta, L. (1998) *Patent Cooperation Treaty (PCT) Int. Appl.* W.O. 98 31219.

[99] Wagner, S.J.; Skripchenko, A., Robinette, D.; Foley, J.W. and Cincotta, L. (1998) *Photochem. Photobiol.*, **67**, 343-349.

[100] Skripchenko, A.A. and Wagner, S.J. (2000) *Transfusion (Bethesda, Md.)*, **40**(8), 968-975.

[101] Wagner, S.; Skripchenko, A. and Friedman, L.I. (2001) *Patent Cooperation Treaty (PCT) Int. Appl.* W.O. 01 49328.

[102] Wagner, S.J. (1991) Patent Cooperation Treaty (PCT) Int. Appl. W.O. 91 16911.

[103] Specht, K.G. (1994) *Photochem. Photobiol.*, **59**, 506-514.

[104] Smelt, D.; Repanovici, R.; Pascaru, A. and Portocala, R. (1976) *Ver. Roum. Med. Virol.*, **27**, 203-207. [*Chem. Abstr.*, **87**, 63405c (1977)].

[105] Lambrecht, B.; Mohr, H., Knuever-Hopf, J. and Schmitt, H. (1991) *Vox Sang.*, **60**, 207-213.

[106] Hiatt, C.W.; Kaufman, E.; Helprin, J.J. and Baron, S. (1960) *J. Immunol.*, **84**, 480-484.

[107] Bellin, J.S.; Lutwick, L. and Jonas, B. (1969) *Arch. Biochem. Biophys.*, **132**, 157-164.

[108] Hassan, H.M. and Fridovich, I. (1979) *Arch. Biochem. Biophys.*, **196**, 385-395.

[109] Martin, J.P. and Logsdon, N. (1987) *Arch. Biochem. Biophys.*, **256**, 39-49.

[110] Singh, H. and Ewing, D.D. (1978) *Photochem. Photobiol.*, **28**, 547-552.

[111] Menezes, S.; Capella, M.A.M. and Caldas, L.R. (1990) *J. Photochem. Photobiol. B*, **5**, 505-517.

[112] Tudek, B.; Laval, J. and Boiteux, S. (1993) *Mol. Gen. Genet.*, **236**, 433-439.

[113] Capella, M.; Coelho, A.M. and Menezes, S. (1996) *Photochem. Photobiol.*, **64**, 205-210.

[114] Menezes, S. and Teixeira, P. (1992) *Int. J. Hyperthermia*, **8**, 689-699.

[115] Capella, M.A.M. and Menezes, S. (1992) *Int. J. Radiat. Biol.*, **62**, 321-326.

[116] Savino, A. and Angeli, G. (1985) *Water Res.*, **19**, 1465-1469.

[117] Mazur, Y.; Acher, A.; Shragina, L. and Avramoff, M. (1993) *U.S. Pat.* 5 220 009.

[118] Millson, C.E.; Buonaccorsi, G.; MacRobert, A.J.; Mlkvy, P. and Bown, S.G. (1995) *Proc. SPIE-Int. Soc. Opt. Eng.*, **2371**, 72-77.

[119] Millson, C.E.; Wilson, M.; MacRobert, A.J. and Bown, S.G. (1996) *J. Photochem. Photobiol. B*, **32**, 59-65.

[120] Koga, T. and Inoue, M. (1981) *Carbohydr. Res.*, **93**, 125-133.

[121] Jacob, H.E. and Hamann, M. (1975) *Photochem. Photobiol.*, **22**, 237-241.

[122] Koenig, K. and Meyer, H. (1992) *Dermatol. Monatsschr.*, **178**, 297-300.

[123] Hill, A.C. (1985) *J. Gen. Microbiol.*, **131**, 181-186.

[124] Gutter, B.; Speck, W.T. and Rosenkranz, H.S. (1977) *Mutat. Res.*, **44**, 177-181.

[125] Epe, B.; Hegler, J. and Wild, D. (1989) *Carcinogenesis (London)*, **10**, 2019-2024.

[126] Shopova, M. and Genov, N. (1977) *Int. J. Pept. Protein Res.*, **10**, 369-374.

[127] Buchnicek, J. (1972) *Acta Fac. Med. Univ. Brun.*, 315-318. [*Chem. Abstr.*, **77**, 109904p (1972)].

[128] Saitow, F. and Nakaoka, Y. (1996) *Photochem. Photobiol.*, **63**, 868-873.

[129] Saitow, F. and Nakaoka, Y. (1997) *Photochem. Photobiol.*, **65**, 902-907.

[130] Lavialle, M. and Dumortier, B. (1978) *Seances Acad. Sci., Ser. D*, **287**, 875-878.

[131] Roth, M.M.M. (1977) *Photochem. Photobiol.*, **25**, 599-600.

[132] Schwartzel, E.H. and Cooney, J.J. (1974) *Can. J. Microbiol.*, **20**, 1015-1021.

[133] Portocala, R.; Sorodoc, G.; Peiulescu, P.; Surdan, C. and Stoian, N. (1972) *Ver. Roum. Virol.*, **9**, 251-252. [*Chem. Abstr.*, **78**, 80373x (1973)].

[134] Sorodoc, G.; Portocala, R.; Peiulescu, P. and Puca, D. (1978) *Ver. Roum. Med. Virol.*, **29**, 233-234. [*Chem. Abstr.*, **89**, 209702n (1978)].

[135] Wilson, M. and Pratten, J. (1994) *Microbios*, **78**, 163-168.

[136] Wilson, M. and Yianni, C. (1995) *J. Med. Microbiol.*, **42**, 62-66.

[137] Wakayama, Y.; Takagi, M. and Yano, K. (1980) *Photochem. Photobiol.*, **32**, 601-605.

[138] Wakayama, Y.; Takagi, M. and Yano, K. (1984) *J. Bacteriol.*, **159**, 527-532.

[139] Bennetto, H.P.; Dew, M.E.; Stirling, J.L. and Tanaka, K. (1981) *Chem. Ind. (London)*, 776-778.

[140] Wilson, M.; Burns, T.; Pratten, J. and Pearson, G.J. (1995). *J. Appl. Bacteriol.*, **78**, 569-574.

[141] Burns, T.; Wilson, M. and Pearson, G.J. (1996) *Proc. SPIE-Int. Soc. Opt. Eng.*, **2625**, 288-297.

[142] Philipp, C.M.; Daskalaki, A.; Algermissen, B. and Weinberg, L. (1999) *8th Congress of the European Society of Photobiology, Granada, Spain, 3-8 September 1999*, Poster 11.

[143] Wainwright, M.; Phoenix, D.A.; Marland, J.; Wareing, D.R.A. and Bolton, F.J. (1997) *F.E.M.S. Immunol. Med. Microbiol.*, **19**, 75-80.

[144] Wainwright, M.; Phoenix, D.A.; Laycock, S.L.; Wareing, D.R.A. and Wright, P.A. (1998) *F.E.M.S. Microbiol. Lett.*, **160**, 177-181.

[145] Wilson, M. and Mia, N. (1993) *J. Oral Pathol. Med.*, **22**, 354-357.

[146] Ito, T. (1977) *Photochem. Photobiol.*, **25**, 47-53.

[147] Ito, T. (1977) *Photochem. Photobiol.*, **25**, 399-401.

[148] Paardekooper, M.; Van der Broek, P.J.A.; De Bruijne, A.W.; Elferink, J.G.R.; Dubbelman, T.M.A.R. and Van Steveninck, J. (1992) *Biochim. Biophys. Acta*, **1108**, 86-90.

[149] Paardekooper, M.; De Bruijne, A.W.; Van Steveninck, J. and Van der Broek, P.J.A. (1993) *Biochim. Biophys. Acta*, **1151**, 143-148.

[150] Paardekooper, M.; De Bruijne, A.W.; Van Steveninck, J. and Van der Broek, P.J.A. (1995) *Photochem. Photobiol.*, **61**, 84-89.

[151] Lazarova, G. and Palazov, D. (1992) *Curr. Microbiol.*, **25**, 31-34.

[152] Takahama, M.S.; Egashira, T. and Takahama, U. (1981) *Photochem. Photobiol.*, **33**, 689-694.

[153] Will III, O.H.; Sawtelle, D.E.; Iverson, P. and Jorve, K. (1988) *Photochem. Photobiol.*, **48**, 305-309.

[154] Ito, T. (1980) *Photochem. Photobiol.*, **31**, 565-570.

[155] Ito, T. (1981) *Photochem. Photobiol.*, **33**, 117-120.

[156] Oseroff, A.R.; Ohuoha, D.; Ara, G.; McAuliffe, D.; Foley, J. and Cincotta, L. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 9729-9733.

[157] Bunting, J.R. (1992) *Photochem. Photobiol.*, **55**, 81-87.

[158] Ball, D.J.; Luo, Y.; Kessel, D.; Griffiths, J.; Brown, S.B. and Vernon, D.I. (1998) *J. Photochem. Photobiol. B*, **42**, 159-163.

[159] Peng, Q.; Brown, S.B.; Moan, J.; Nesland, J.M.; Wainwright, M.; Griffiths, J.; Dixon, B.; Sawyer, J.C. and Vernon, D. (1993) *J. Photochem. Photobiol. B*, **20**, 63-71.

[160] Gill, W.B.; Huffman, J.L.; Lyon, E.S.; Bagley, D.H.; Schoenberg, H.W. and Straus II, F.G. (1984) *Cancer*, **53**, 2724-2727.

[161] Kaisary, A.V. (1986) *Urology*, **28**, 100-102.

[162] Gill, W.B. and Straus, F.G. (1984) *Urology Suppl.*, 63-66.

[163] Vicent, J.; Chechile, G. and Algaba, F. (1987) *Eur. Urol.*, **13**, 15-16.

[164] Rück, A.; Rimmele, E.S. and Steiner, R. (1998) *Ger. Offen D.E.* 19640722 A1.