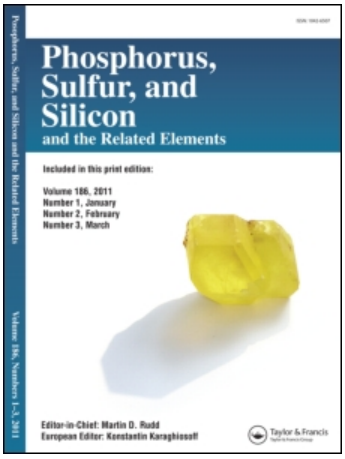


Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

Photosensitizing Merocyanine Dyes Based on Selenobarbituric Acid

Wolfgang H. H. Günther[a]; Roger Searle[a]; Fritz Sieber[b]
[a] Sterling Drug Inc., Malvern, PA, USA [b] Medical College of Wisconsin, Milwaukee, WI, USA

To cite this Article Günther, Wolfgang H. H. , Searle, Roger and Sieber, Fritz(1992) 'Photosensitizing Merocyanine Dyes Based on Selenobarbituric Acid', Phosphorus, Sulfur, and Silicon and the Related Elements, 67: 1, 417 — 424
To link to this Article: DOI: 10.1080/10426509208045864
URL: http://dx.doi.org/10.1080/10426509208045864

Phosphorus, Sulfur, and Silicon, 1992, Vol. 67, pp. 417–424
Reprints available directly from the publisher
Photocopying permitted by license only

PHOTOSENSITIZING MEROCYANINE DYES BASED ON SELENOBARBITURIC ACID

WOLFGANG H.H. GÜNTHER*, ROGER SEARLE*, AND FRITZ SIEBER**
*Sterling Drug Inc., Malvern, PA, 19355-1314, USA
**Medical College of Wisconsin, Milwaukee, WI, 53226, USA

Summary: Structural analogs of Merocyanine 540 (MC540) were synthesized to probe biological mechanisms of action and to enhance viral inactivation. Novel pyrimidine-2-selone analogs of known and modified merocyanine sensitizers are described. A surprising result of placing selenium at the barbiturate site was the ~100-fold enhancement of photogeneration efficiency for singlet oxygen ($^1O_2$). Conversely, selenium directly attached to the chromophore π-system was much less efficacious. The production of more $^1O_2$ is reflected in greatly improved biological effectiveness of the new dyes. A puzzling high to low bioactivity isomer effect among molecules containing naphthoxazole or naphthothiazole components is shown by both thione and selone dyes.

Introduction

Merocyanine 540 and related N-sulfoalkyl merocyanine dyes derived from 1,3-dialkyl-2-thiobarbituric acid (Fig. 1 and Table 1) are photosensitizers for the light-induced killing of leukemia cells and enveloped viruses. The latter include the human pathogens HIV, HTLV-l, CMV, HSV, and the hepatitis viruses HBV and NANB (or HCV). The antiviral effect offers an opportunity for the prophylaxis of blood and blood products in order to decrease the risk of infection from transfusion.

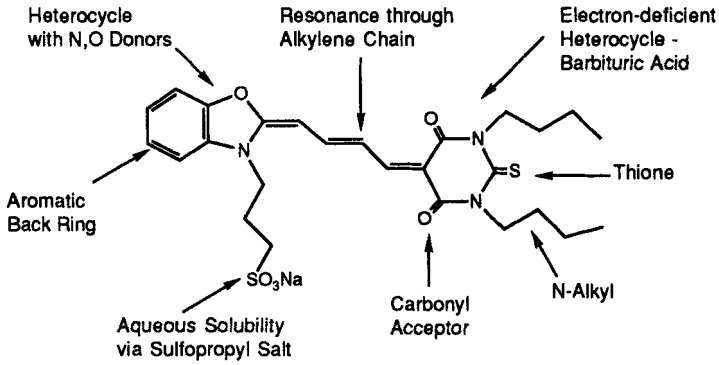
The biocidal activity of photo-excited MC540 has been demonstrated to derive from the dye-sensitized formation of singlet oxygen, a highly active oxidizing agent.[1,2,3] However, it has also been reported that the quantum yield for singlet oxygen generation sensitized by MC540 is only 0.007.[4] We have found similarly low values for a novel series of dye analogs.[5]

The activity of merocyanine sensitizers against herpes simplex virus *in vitro* depends strongly on the structure of the dye (see Fig. 1). For instance, activity is greatly reduced or abolished whenever the barbiturate N-substituents are made more polar. This holds for polar substituents, -H or $-CH_2CH_2OCH_3$, or even a short alkyl, N-ethyl, replacing the N-butyl groups. Changing the chalcogen at the 2-position of the barbituric acid moiety from sulfur to oxygen also reduces activity. These effects do not result from changes in spectra nor from changes in photogeneration quantum efficiency. They are qualitatively related to reduced oleophilicity, although our efforts to determine distribution coefficients between water and octanol were frustrated by the exceedingly low concentrations of the dyes in the aqueous phase.

Turning the direction of these changes around proved advantageous. An expansion of the aromatic back ring to naphthalene produced a significant enhancement in biological activity for two of the three isomers and a puzzling decrease for dyes containing naphth[1,2-d]-oxazole or -thiazole moieties. Lengthening the barbiturate N-substituents

much beyond n-butyl or branching them proved unprofitable. The syntheses became much less efficient with lengthy reaction times and lower yields. Worse, the resulting dyes had reduced solubilities in biologically acceptable aqueous media.[5]

FIG. 1: Merocyanine 540
Anatomy of a Sensitizing Dye



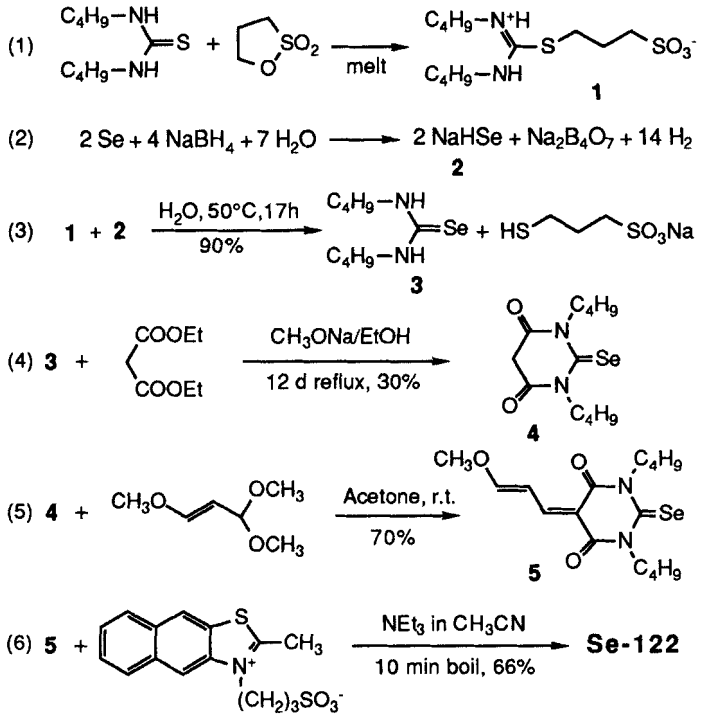
However, the significant step in activity between oxygen and sulfur substituents suggested that further enhancements might be obtained if sulfur, in turn, were to be replaced by selenium, the next higher chalcogen homolog in the periodic table of elements. Such seleno-substituted merocyanine dyes were not known in the chemical literature.

Isosteric, non-isoelectronic replacement of sulfur by selenium has been used in the past to probe mechanistic aspects of sulfur functionality in a biological context.[6] Specifically of interest to us was the single reported synthesis of 5-substituted 2-seleno-barbiturates by Mautner and Clayton in 1959.[7] The major finding for these analogs of barbiturate hypnotics was an increase in lipid solubility as the 2-substituent was changed from oxygen to sulfur and further to selenium. Mautner and Clayton's compounds lack alkyl substituents on the barbiturate nitrogen atoms but they demonstrated that 2-selenobarbiturates are stable compounds. Therefore, the analogous 1,3-dialkyl-2-selenobarbituric acids should be accessible as source materials for the desired syntheses of dyes that are direct analogs of our most promising sensitizers based on 1,3-dialkyl-2-thiobarbiturates.

Syntheses

The synthetic route of Scheme 1 was found to be readily applicable to the dyes under consideration. To prepare the 1,3-dialkyl-2-selenourea, a modification of the method of Klayman and Shine[8] was employed. Thus, 1,3-dibutyl-2-thiourea was S-alkylated using 1,3-propanesultone (Step 1), followed by a nucleophilic displacement reaction with sodium hydrogen selenide in aqueous solution (Step 3). The sodium hydrogen selenide reagent was generated by reduction of elemental selenium with sodium borohydride in water (Step 2). The use of a water-soluble and non-volatile leaving group such as 3-mercaptopropanesulfonic acid has an advantage over the usual procedure employing simple alkyl groups in which highly volatile, odoriferous mercaptoalkanes are released.

Scheme 1

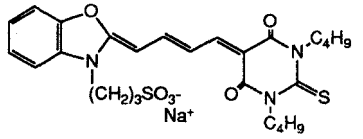
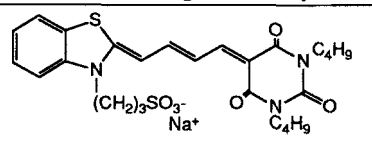
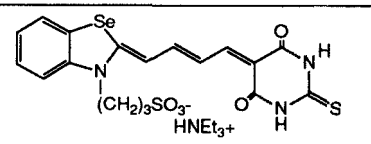
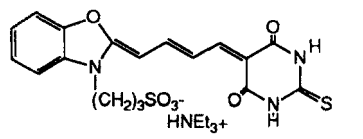
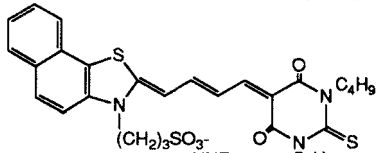
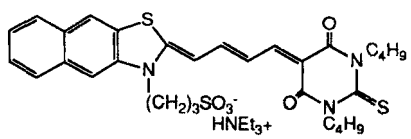
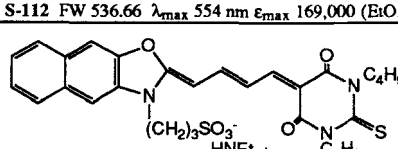
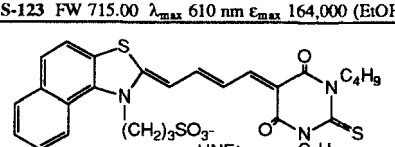
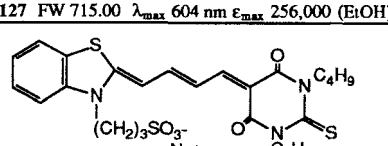
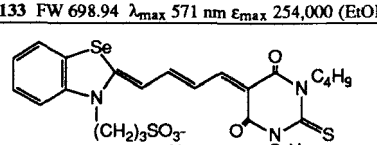
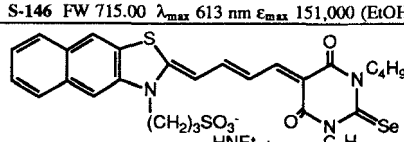
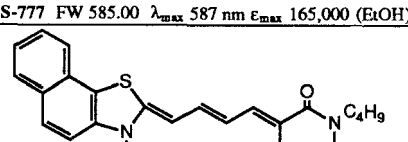
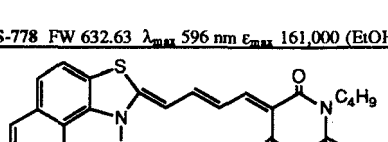
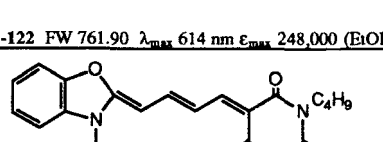
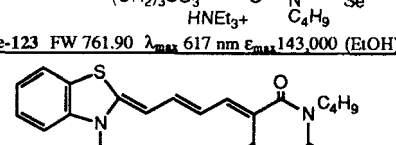


Condensation of 1,3-dibutyl-2-selenourea with diethyl malonate in alcoholic solution in the presence of sodium methylate (Step 4) proceeded very slowly. Workup after heating under reflux for 12 days resulted in recovery of about one half of the initial selenourea and a 15% yield of crystalline 1,3-dibutyl-2-selenobarbituric acid. Another 15% was recovered by converting the mother liquors to 1,3-dibutyl-2-seleno-4,6-diketo-5-(3'-methoxypropenylidine)pyrimidine (Step 5). Condensation of the latter with appropriate 2-methyl-3-sulfopropyl quaternary salts in the presence of triethylamine (Step 6), followed by crystallization of the products yielded the Se-dyes depicted in Table 1.

Physical and Photochemical Properties

The absorption spectra of the selone dyes were similar to those of their thione analogs; they showed only small bathochromic shifts in maxima and slight broadening of the bands. The substitution does not formally involve the chromophore; no simple valence bond resonance structures can be written that include the chalcogen atoms at the 2-position of the barbituric acid moiety.

TABLE 1: Numerical Listing of Merocyanine Dyes

 *MC540* FW 569.67 λ_{max} 560 nm ε_{max} 179,000 (EtOH)	 *S-053* FW 569.67 λ_{max} 574 nm ε_{max} 185,000 (EtOH)	 *S-108* FW 599.62 λ_{max} 596 nm ε_{max} 177,000 (EtOH)
 **S-112** FW 536.66 λ_{max} 554 nm ε_{max} 169,000 (EtOH)	 **S-123** FW 715.00 λ_{max} 610 nm ε_{max} 164,000 (EtOH)	 **S-127** FW 715.00 λ_{max} 604 nm ε_{max} 256,000 (EtOH)
 **S-133** FW 698.94 λ_{max} 571 nm ε_{max} 254,000 (EtOH)	 **S-146** FW 715.00 λ_{max} 613 nm ε_{max} 151,000 (EtOH)	 **S-777** FW 585.00 λ_{max} 587 nm ε_{max} 165,000 (EtOH)
 **S-778** FW 632.63 λ_{max} 596 nm ε_{max} 161,000 (EtOH)	 **Se-122** FW 761.90 λ_{max} 614 nm ε_{max} 248,000 (EtOH)	 **Se-123** FW 761.90 λ_{max} 617 nm ε_{max} 143,000 (EtOH)
 **Se-124** FW 761.90 λ_{max} 618 nm ε_{max} 125,000 (EtOH)	 **Se-125** FW 616.57 λ_{max} 567 nm ε_{max} 150,000 (EtOH)	 **Se-126** FW 632.63 λ_{max} 602 nm ε_{max} 153,000 (EtOH)

The sensitized production of singlet oxygen entails three steps: (1) photoexcitation of the sensitizer to the excited singlet state; (2) intersystem crossing of the excited sensitizer from the singlet to the triplet state; and (3) energy transfer from sensitizer to a ground state oxygen molecule to give ground state sensitizer and singlet excited oxygen. It has long been recognized that molecules containing atoms of high atomic number undergo step (2) at higher rates than do close analogs containing no "heavy atoms."

Diphenylisobenzofuran (DPIBF) is an excellent scavenger for singlet oxygen. Under the appropriate conditions, the photosensitized oxidation of DPIBF follows first order kinetics with a rate constant directly proportional to the quantum yield for singlet oxygen production.[9] We have used this procedure to obtain relative singlet oxygen quantum yields.

Substitution of selenium for oxygen was somewhat effective in enhancing singlet oxygen production when the atom replacement occurred in the oxazole ring. Dye S-778 had five times the quantum efficiency of MC540, and S-108 had seven times the quantum efficiency of S-112. In both cases, corrections for the relative amount of light absorbed were made; the selenium is conjugated with the chromophore and causes a substantial bathochromic shift in the absorption spectrum. In accordance with these results, we found S-778 to be about twice as effective as MC540 in biocidal activity (see Table 2).

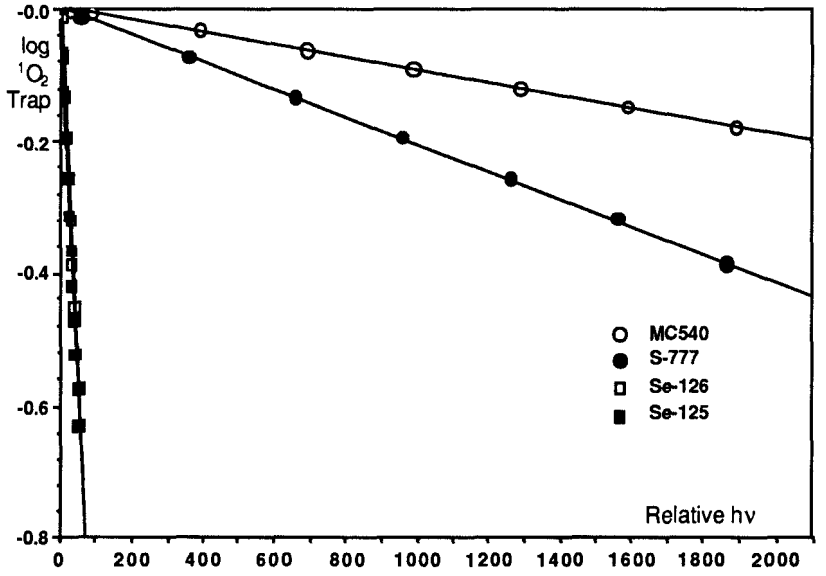


FIG. 2. $^1O_2$ Generation from Thione and Selone Dyes

The DPIBF method was then used to compare merocyanine dyes derived from 1,3-dibutyl-2-selenobarbituric acid to dyes based on the corresponding thiobarbituric moiety. The absorption spectra were sufficiently similar that corrections for unequal absorption of the light were not necessary. We found, surprisingly, that Se-125 had 120 times the quantum efficiency of MC540 and Se-126 had 60 times the quantum efficiency of the thio analog, S-777 (Fig. 2). Further, Se-123 had 74 times the quantum efficiency of S-123. On the basis of the enhanced oxygen activation, it might be predicted that the seleno analogs would also show greater singlet oxygen mediated biocidal activity.

Anti-viral Effects

The anti-viral performance of the selone dyes (Table 2) was assessed against comparison dyes originating from 2-thiobarbituric acid. Dyes from an appropriate stock solution in 50% aqueous ethanol were added to viral cultures of herpes simplex Type 1. The molar dye concentrations were held constant within each experiment. An initial titer of the number of viral infective units (plaque-forming units = PFU) was determined by a limiting dilution plaque-forming assay on Vero cells. The initial absolute count of PFU/ml of viral culture was generally between about 10^5 to 10^7 units.

The cultures were illuminated with light from a bank of cool white fluorescent tubes at an intensity ranging from about 35 watts/m^2 to 70 watts/m^2. Aliquots of the cultures were periodically removed over a 10 minute period and assayed. For purposes of expressing test results, the initial PFU count was set to unity and used as the basis to calculate the reduction due to the treatment.

Each of the experiments in Table 2 represents a comparison study between a small group of sensitizing dyes, i.e., the relative effectiveness of materials under the directly comparable and controlled conditions of a single experiment. Results are tabulated as the logarithms of the ratios between initial PFUs and those found in the limiting dilution assay (log reduction values). In order to appreciate the magnitude of the anti-viral effects achieved, it must be understood that each 1.0 log unit difference represents a ten-fold step in number of PFUs counted, i.e., -6.0 logs equates to a million-fold reduction of the infective population.

Expt. 1 lists the series wherein the oxygen atom in benzoxazole was successively replaced by sulfur and then by selenium. It can be seen that there is a progressive enhancement in activity. Expt. 2 lists MC540 and an analogous dye based on naphtho-[2,3-d]thiazole. The enhancement in activity exceeds that found for the progressive chalcogen displacement in Expt.1. Expt. 3 compares properties of the remaining two naphthothiazoles. Surprisingly, naphtho[1,2-d]thiazole was rather inactive and naphtho-[2,1-d]thiazole was so active that the viral count at the 8 and 10 min times fell below detectable limits. This large difference between the isomers is remarkable because the quantum yield for singlet oxygen generation (DBIBF method) from S-146 was 0.81 that determined for S-123. It is seen that merocyanine dyes derived from 1,3-dibutyl-2-thio-barbituric acid have anti-viral activities ranging from less than 1 log unit to complete kill under the conditions of the experiment.

Expt. 4 illustrates the effectiveness of the new selenomerocyanine dyes by comparison to the thiobarbiturate reference compounds. The log reduction values for compound S-123 indicate that the experimental conditions remained reproducible, such that only the 8 and 10 minute data points were below detectable limits. For two of the selenobarbiturate dyes no viral infective units could be detected even at the shortest photoexposure time of 2 minutes. Activity of Se-124 was reduced (in analogy to S-146, Expt.3). Still, the viral reduction at the 2 and 4 minute points was about 2 log units greater than that achieved by the comparison compound S-123.

The detectability of viral inactivation was improved for Expt. 5 by providing an extremely heavy inoculum, i.e., the virus titer was enhanced to 1.5 x 10^7 PFU/ml by centrifugation of the virus stock. For the comparison compound S-123 the two additional data points at the 8 and 10 minute level illustrate the expected steady progression of viral inactivation. The activity of the benzoxazole compound, Se-125 (selone analog of MC540), exceeded that of the comparison at all data points. The benzothiazole dye, Se-126, had an activity equivalent to Se-125 at the 2 minute exposure point, but then there was, essentially, no further progression for the remainder of the experiment.

Table 2: Antiviral Effectiveness of Selenomerocyanines

Virus	: Human herpes simplex, type 1
Virus Titer	: 6.5 x 10^5 PFU/ml, approximately
Dye concentration	: see Table (added from stock solution in 50% ethanol)
Serum	: Fetal bovine serum, 14%
Medium	: HEPES-buffered (10 mM, pH 7.4) alpha-medium
Light	: Cool white fluorescent, see Table for W/m^2
Assay	: Plaque formation, Vero cells 3 S
Data	: Expressed as log depletion of initial titer.

Experimental Conditions	Dye	Minutes Exposure 2	4	6	8	10
Expt. 1: Thione Dyes 3.3 µM dye, ~70 W/m^2	MC540	-0.52	-0.96	-1.22	-2.00	-2.57
	S-777	-1.26	-1.89	-2.40	-2.82	-3.22
	S-778	-1.48	-2.12	-2.66	-3.52	-4.01
Expt. 2: Thione Dyes 3.3 µM dye, ~70 W/m^2	MC540	-0.43	-0.72	-1.30	-1.52	-2.00
	S-127	-1.37	-3.00	-4.15	-4.40	-4.77
Expt. 3: Thione Dyes 3.3 µM dye, ~70 W/m^2	S-146	-0.26	-0.35	-0.49	-0.60	-0.77
	S-123	-3.22	-3.52	-4.12	*	*
Expt. 4: Selone Dyes 3.3 µM dye, ~70 W/m^2	S-123	-3.40	-4.05	-4.46	*	*
	Se-122	*	*	*	*	*
	Se-124	-5.10	-6.10	*	*	*
	Se-123	*	*	*	*	*
Expt. 5: Selone Dyes 3.3 µM dye, ~70 W/m^2	S-123	-3.35	-4.12	-4.82	-5.37	-6.22
	Se-125	-4.37	-4.72	-5.52	*	*
	Se-126	-4.43	-4.49	-4.74	-4.74	-4.82
Expt. 6: Selone Dyes 0.65 µM dye, ~70 W/m^2	S-123	-1.12	-1.89	-2.49	-3.05	-3.47
	Se-122	-4.10	-4.30	-4.35	-4.40	-4.46
	Se-123	-3.64	-3.70	-3.70	-4.35	-4.46
	Se-124	-1.40	-1.82	-2.46	-2.82	-2.92
	Se-125	-1.18	-1.92	-3.02	-3.07	-3.12
	Se-126	-1.74	-1.92	-2.02	-2.07	-2.12
Expt. 7: Selone Dyes 0.88 µM dye, ~35 W/m^2	S-123	-0.70	-1.00	-1.10	-1.52	-1.74
	Se-122	-2.00	-2.82	-3.40	-4.52	-5.60
	Se-123	-1.74	-2.89	-3.57	-4.26	-4.30
Expt. 8: Selone Dyes 0.88 µM dye, ~35 W/m^2 30 min pre-incubation	S-123	-0.98	-1.10	-1.52	-1.52	-2.05
	Se-122	-2.10	-3.05	-4.60	-6.30	*
	Se-123	-1.70	-2.00	-2.70	-3.00	-3.30

* Viral count below detectable limit

Expt. 6 illustrates the effect of a 5-fold reduction in dye concentration for the set. It is seen that all of the new dyes match or exceed the activity of the comparison compound, S-123, at the 2-minute exposure time. At this time point Se-122 exceeds the viral inactivation due to S-123 by 3 logs (1000-fold). However, Se-122, Se-123, and Se-126 then do not show a further log/linear progression of viral inactivation over the time-course of the experiment. Se-124 and Se-125 show a time-course that roughly parallels that of the comparison compound. It has been shown, in similar experiments by us and others,[10] that residual cells or viruses are not resistant strains. It is possible that bleaching of the highly dilute selenomerocyanine dye solutions under illumination conditions is responsible. The curve shape can also be modeled by assuming incomplete equilibration of the dye through the target population: a few virus particles remain unstained under the conditions of the experiment and escape photodestruction. Some support for the importance of proper diffusion is provided by slowing the entire process (35 W/m^2) and by including a relatively lengthy (30 min) pre-incubation period (Expt. 8). Under these conditions the viral count for the Se-122 experiment at 10 min fell below the 7 log detectable limit.

Conclusions

We have demonstrated that merocyanine dyes that contain a selone function not directly attached to the dye resonance π-system exhibit near unit efficiency for the photogeneration of singlet oxygen. This ~100-fold photophysical improvement over equivalent thione dyes translates into a significant enhancement of photobiological activities against herpes simplex, a model for enveloped virus pathogens. The process for viral inactivation is simple, requiring photoexposures on the order of a few minutes at light levels readily achievable with commercial fluorescent light sources. Because dyes with equivalent photogeneration efficiencies may show vastly different biological photoeffects, the requirement for physical invasion of the organism appears to be demonstrated. On the basis of our observations with the naphtho isomers, we are also tempted to speculate that the dyes bind to a site on the virus that has a significant stereochemical selectivity.

References

1 B. Kalyanaraman, J. B. Feix, F. Sieber, J. P. Thomas and A. W. Girotti, *Proc. Nat. Acad. Sci.* USA **84** (9), 2999-3003 (1987)

2 D. P. Valenzeno, J. Trudgen, A. Hutzenbuhler, and M. Milne, *Photochem. Photobiol.* **46** (6) 985-990 (1987)

3 J. B. Feix, B. Kalyanaraman, C. F. Chignell, and R. D. Hall, *J. Biol. Chem.* **263** (33) 17247-17250 (1988)

4 M. Hoebeke, A. Seret, J. Piette, and A. van de Vorst, *J. Photochem. Photobiol., B: Biology* **1**, 437-446 (1988)

5 W. H. H. Günther, F. Sieber, and R. Searle, *Photomedicine Symposium* at 20th Annual Meeting, International Society for Experimental Hematology, Parma, Italy, July 22-26, 1991.

6 For examples see: *Organic Selenium Compounds, Their Chemistry and Biology*, D. L. Klayman and W. H. H. Günther, Editors. John Wiley & Sons, Inc 1973. ISBN 0-471-49032-6; see especially chapters XId, XII, XIII.

7 H. G. Mautner and E. M. Clayton, *J. Amer. Chem. Soc.*, **81**, 6270 (1959)

8 D. L. Klayman and R. J. Shine, *J. Org. Chem.* **34**, 3549 (1969)

9 R. H. Young, K. Wehrly, and R. L. Martin, *J. Amer. Chem. Soc.*, **93** (22) 5774 (1971)

10 C. Pfau, Rensselaer Polytechnic Inst., personal communication.