Synthesis and Evaluation of Chalcogenopyrylium Dyes as Potential Sensitizers for the Photodynamic Therapy of Cancer

Kristi A. Leonard,[†] Marina I. Nelen,[†] Todd P. Simard,[†] Sherry R. Davies,[§] Sandra O. Gollnick,[§] Allan R. Oseroff,[§] Scott L. Gibson,[‡] Russell Hilf,[‡] Lan Bo Chen,[||] and Michael R. Detty*[,†]

Departments of Chemistry and Medicinal Chemistry, State University of New York at Buffalo, Buffalo, New York 14260, Department of Biochemistry and Biophysics, University of Rochester Medical Center, 601 Elmwood Avenue, Box 607, Rochester, New York 14642, Department of Dermatology and PDT Center, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, New York 14263, and Dana-Farber Cancer Institute, Harvard Medical School, 44 Binney Street, Boston, Massachusetts 02115

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A series of thiopyrylium (**2**), selenopyrylium (**3**), and telluropyrylium dyes (**4**) was prepared via the addition of Grignard reagents to either 2,6-di(4-dimethylamino)phenylchalcogenopyran-4-ones (**5a**) or 2-[4-(dimethylamino)phenyl]-6-phenylchalcogenopyran-4-ones (**5b**) followed by elimination and ion exchange to give the chloride salts. The absorption spectra and quantum yields for singlet oxygen generation of these dyes suggested that the dyes would have utility as sensitizers for PDT. Selenopyrylium dyes **3a** and **3d** with quantum yields for singlet oxygen generation of 0.040 and 0.045, respectively, were phototoxic to Colo-26 cells in culture. The toxicity of the dyes **2**−**4** was evaluated in clonogenic assays of human carcinoma cell lines. Importantly, the presence of a sulfur, selenium, or tellurium heteroatom in the molecules had no predictable impact on the toxicity of any particular dye set. Substituents at the 2-, 4-, and 6-positions of the dye had a much greater impact on cytotoxicity. The IC_{50} values determined in the clonogenic assays did not correlate with chemical properties in the dye molecules such as reduction potential or lipophilicity. Initial in vivo toxicity studies showed no toxicity for these dyes at dosages between 7.2 and 38 μmol/kg in BALB/c mice.

Introduction

Photodynamic therapy (PDT) is a relatively recent development in cancer therapy that has recently won regulatory approval in the United States, Canada, The Netherlands, France, Germany, and Japan for cancers of the lung, digestive tract, and genitourinary tract.[1] As a therapy, PDT uses a light-activated sensitizer to produce a cytotoxic reagent or a cytotoxic reaction in the tumor cell, typically via generation of singlet oxygen or superoxide from molecular oxygen.[1a] Photofrin, a mixture of porphyrins derived from hematoporphyrin, has received regulatory approval for use in PDT, but the Photofrin mixture is not an ideal sensitizer. Photofrin and other porphyrin derivatives are only weakly absorbing at wavelengths of light longer than 600 nm, where penetration of light in tissue is optimal.

Cationic dyes have been explored as sensitizers for PDT, and the classes explored include the Victoria blue dyes,[2] methylene blue[3] and the related nile blue dyes,[4] cyanine dyes,[5] rhodacyanine dyes,[6] and telluropyrylium dyes.[7] These classes of dyes have absorption maxima of greater than 600 nm and have molar extinction coefficients of $\geq 10^4$ M^{-1} cm^{-1}, which makes them more effective at harvesting light during PDT. Many of the cationic dyes accumulate selectively in transformed cells relative to normal cells with the mitochondria as important cellular targets.[2,3,5−7]

The cationic sensitizers for PDT share structural features with several classes of π-electron-delocalized, lipophilic, cationic compounds (DLCs) that have been evaluated as selective anticarcinoma agents. DLCs such as rhodamine 123 (Rh-123),[8] dequalinium chloride,[9] thiacarbocyanines,[10] Victoria blue BO (also a sensitizer for PDT),[2a] tetraphenylphosphonium,[11] rhodacyanines,[12,13] and the thiopyrylium dye AA1[14] show selective toxicity toward carcinoma cells relative to normal epithelial cells. Like the cationic sensitizers for PDT, many of the DLCs are targeted to the mitochondria of cells presumably in response to the high negative charge in the mitochondria. It is hypothesized that the difference in transmitochondrial membrane potentials between normal epithelial cells and carcinoma cells may be responsible for the increased uptake and prolonged retention of cationic dyes and other DLCs in carcinoma cells.[8,15]

Even though they accumulate selectively in tumors in vivo, the chalcogenopyrylium dyes Rh-123[8] and AA1[14] are poor sensitizers for PDT presumably due to their low quantum yields for singlet oxygen generation. Earlier studies with the chalcogenopyrylium dye series **1** (Chart 1) demonstrated that the heteroatom impacts the rate of intersystem crossing to the triplet and, consequently, the quantum yield for singlet oxygen generation.[2a,7] Those analogues incorporating at least one selenium or tellurium atom are useful as PDT sensitizers. By analogy, analogues of Rh-123 and AA1 incorporating the heavier chalcogen atoms should be more efficient sensitizers for PDT. We have recently described the synthesis of thiopyrylium dye **2a**, selenopyrylium dye **3a**, and telluropyrylium dye **4a** (Chart

* To whom correspondence should be addressed.
[†] State University of New York at Buffalo.
[‡] University of Rochester Medical Center.
[§] Roswell Park Cancer Institute.
[||] Dana-Farber Cancer Institute.

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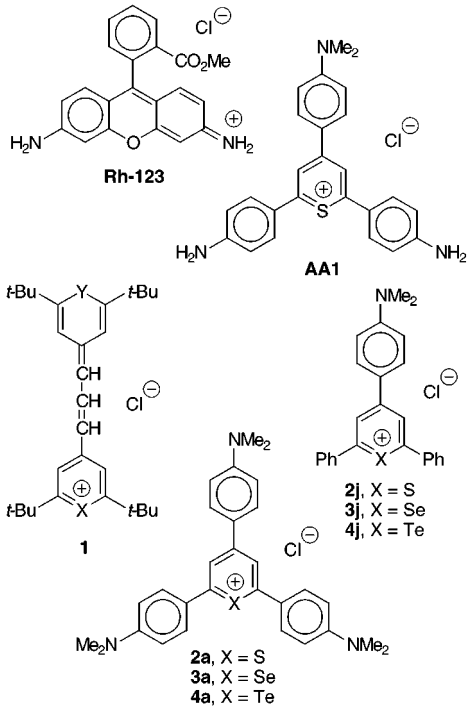
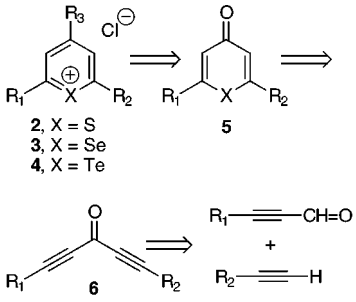
Chart 1



Scheme 1



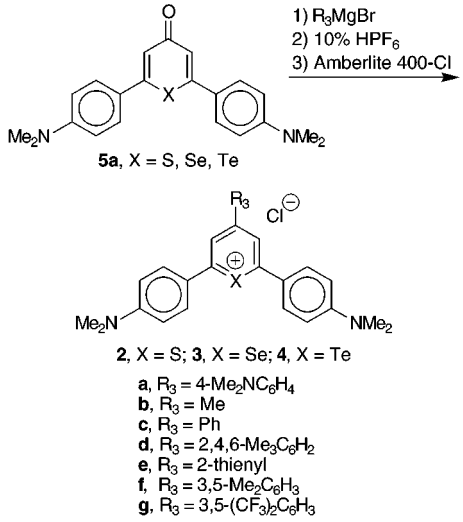
1) as analogues of the AA1 structure and have demonstrated that all three dyes are photosensitizers in vitro.[16] The synthetic routes to **2a**−**4a** also permit the incorporation of a variety of different substituents around the π-framework in addition to the heteroatom changes in the chalcogenopyrylium ring.

In this article, we describe the synthesis of a series of dyes related in structure to AA1 with varied substituents at the 2-, 4-, and 6-positions of the thiopyrylium (**2**), selenopyrylium (**3**), and telluropyrylium (**4**) nucleus. The effects of structure on chemical and photophysical properties important to PDT sensitizers (absorption maxima, reduction potentials, quantum yields for singlet oxygen generation) were evaluated. The effects of heteroatom substitution in the chalcogenopyrylium ring on toxicity were evaluated in vitro with a clonogenic assay and in vivo with BALB/c mice.

Chemical Results and Discussion

Synthesis. The thio-, seleno-, and telluropyrylium dyes related to AA1 were prepared by a common synthetic approach as shown in the retrosynthesis of Scheme 1. The addition of Grignard reagents or organolithium compounds to the carbonyl carbon of chalcogenopyranones **5** followed by acid-induced elimination of water leads to chalcogenopyrylium dyes **2**−**4** with a

Scheme 2

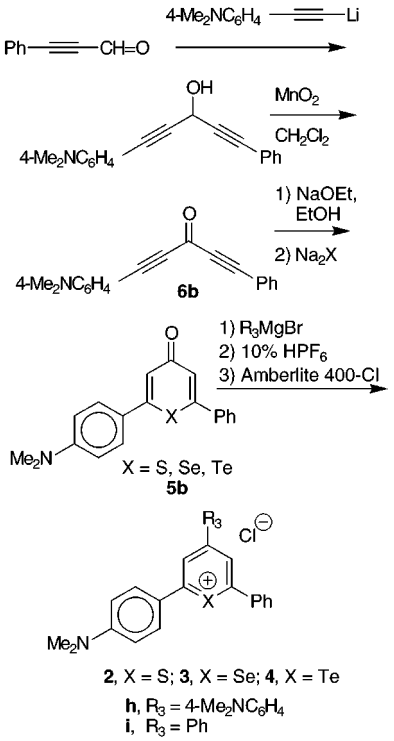


variety of different groups at the 2-, 4-, and 6-positions.[17,18] Thiopyranones, selenopyranones, and telluropyranones **5** (X = S, Se, Te) can be prepared from 1,4-pentadiyn-3-ones **6** as a common intermediate through the formal addition of hydrogen sulfide, selenide, and telluride, respectively.[18] Unsymmetrical diynones **6** ($R_1 \neq R_2$) are prepared by the formal coupling of a terminal acetylene with an alkynyl aldehyde,[19] while symmetrical diynones **6** ($R_1 = R_2$) are prepared by the formal coupling of 2 equiv of a terminal acetylene and formaldehyde.[17,20]

2,6-Di[4-(dimethylamino)phenyl]chalcogenopyran-4-ones **5a** were prepared by the addition of disodium chalcogenides to 1,5-di[4-(dimethylamino)phenyl]-1,4-pentadiyn-3-one (**6a**).[16] Dyes **2a**−**4a** were prepared by the addition of 4-(dimethylamino)phenylmagnesium bromide to chalcogenopyranones **5a** (Scheme 2).[16] The intermediate alcohols were dehydrated with cold 10% HPF_6, and the hexafluorophosphate salts were exchanged for chloride on an ion-exchange resin. Following the same sequence with methylmagnesium bromide gave dyes **2b**−**4b** in 68−74% isolated yields; with phenylmagnesium bromide, dyes **2c**−**4c** in 51−80% isolated yields; with mesitylmagnesium bromide, dyes **2d**−**4d** in 45−75% isolated yields; with 2-thienylmagnesium bromide, dye **4e** in 67% isolated yield; with 3,5-dimethylphenylmagnesium bromide, dyes **3f** and **4f** in 62% and 67% isolated yields, respectively; and with 3,5-di(trifluoromethyl)phenylmagnesium bromide, dyes **3g** and **4g** in 74% and 51% isolated yields, respectively.

Unsymmetrical dyes **2h**−**4h** and **2i**−**4i** were prepared as shown in Scheme 3 from 1-[4-(dimethylamino)-phenyl]-5-phenyl-1,4-pentadiyn-3-one (**6b**) as a common intermediate. The addition of lithium 4-(dimethylamino)phenylacetylide[20] to phenylpropargyl aldehyde followed by oxidation of the intermediate diynol with manganese dioxide gave diynone **6b** in 67% overall yield. 2-[4-(Dimethylamino)phenyl]-6-phenylchalcogenopyran-4-ones **5b** were prepared in two steps from **6b**. The initial step converted **6b** to a mixture of enol ethers through the addition of ethanol across one triple bond in 0.2 N NaOEt in ethanol.[16] The enol ether mixture was then added to solutions of disodium chalcogenides to give chalcogenopyranones **5b** (X = S, Se, Te) in 67−73% isolated yields. The addition of 4-(dimethylamino)-

Scheme 3

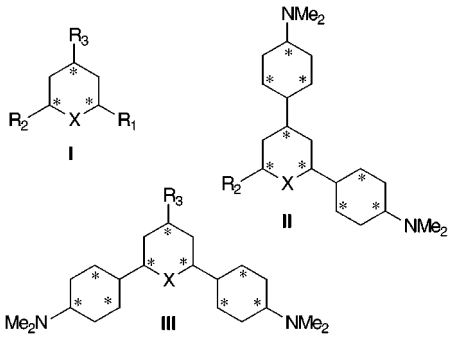


phenylmagnesium bromide and phenylmagnesium bromide, respectively, to chalcogenopyranones **5b** followed by dehydration with 10% HPF_6 and ion exchange gave dyes **2h**−**4h** and **2i**−**4i**.

The presence of a nitrogen-containing functionality appears to be important in cationic PDT sensitizers and in DLCs that show either selective uptake in carcinoma cells or selective toxicity toward carcinoma cells. Both AA1[14] and the Victoria blue dyes[2] have three nitrogen-containing functional groups while Rh-123,[8] methylene blue[3] and the related nile blue dyes,[4] cyanine dyes,[5] thiacarbocyanines,[10] rhodacyanine dyes,[6] and dequalinium salts[9] have two. The dye series **2**−**4** incorporates some systematic changes in the number and placement of the nitrogen-containing functionality. Dyes **2a**−**4a** place three 4-(dimethylamino)phenyl substituents at the 2-, 4-, and 6-positions of the chalcogenopyrylium nucleus. Dyes **2c**−**4c** and **2h**−**4h** place two 4-(dimethylamino)-phenyl substituents at the 2- and 6-positions and at the 2- and 4-positions, respectively. Dyes **2i**−**4i** and **2j**−**4j**[21] (Chart 1) place one 4-(dimethylamino)phenyl substituent at either the 2- or 4-position, respectively. Dyes **2b**−**4b** through dyes **2g**−**4g** have two 4-(dimethylamino)-phenyl substituents at the 2- and 6-positions as a common structural feature while varying the substituents attached to the 4-position, which include alkyl, aryl, and heteroaryl groups.

Absorption Spectra of Dyes 2−4. For the dyes **2**−**4** to be useful as sensitizers for PDT, they must absorb light of greater than 600 nm and produce a cytotoxic species such as singlet oxygen upon irradiation.[22] The depth of nonthermal penetration of light in tissue is greatest between 600 and 900 nm. At longer wavelengths, absorption of light by the infrared harmonics of water both generates heat during excitation and reduces the fraction of light reaching the sensitizer, while at shorter wavelengths, absorption by biological chromophores produces similar effects.[23]

Chart 2



Earlier studies have shown that heteroatom substitution affects absorption maxima (λ_{max}), with λ_{max} increasing in the following order: pyrylium < thiopyrylium < selenopyrylium < telluropyrylium dyes.[7,21] The thiopyrylium dyes of this study have values of λ_{max} between 592 nm for **2j** and 675 nm for **2c**. The corresponding selenopyrylium analogues **3** absorb at longer wavelengths (620−723 nm) and the telluropyrylium analogues **4** at yet longer wavelengths (653−771 nm), which is consistent with the earlier studies. All of the dyes **2**−**4** prepared in this study absorb light of appropriate wavelengths useful for PDT.

The chalcogenopyrylium nucleus when drawn as an alternating Hückel π-system has starred carbons at the 2-, 4-, and 6-positions as shown in structure **I** of Chart 2. Following empirical rules, electron-donating substituents attached to the starred carbons should give shorter wavelengths of absorption while electron-withdrawing substituents should give longer absorption maxima.[21] The dyes **2a**−**2d**, **3a**−**3g**, and **4a**−**4g** have two 4-(dimethylamino)phenyl substituents at the 2- and 6-positions but vary the substituent in the 4-position. In comparisons of λ_{max} for these dyes, dyes with a 4-substituent more electron-donating than phenyl have shorter values of λ_{max} [4-(dimethylamino)phenyl, methyl, mesityl, 3,5-dimethylphenyl] than dyes with a 4-substituent more electron-withdrawing than phenyl [2-thienyl, 3,5-di(trifluoromethyl)phenyl].

While the 4-(dimethylamino)phenyl substituents are electron-donating relative to a phenyl substituent, the nitrogen atoms help delocalize the positive charge and give cyanine character to the dye chromophores. Two 4-(dimethylamino)phenyl substituents can be placed at the 2,4-positions or at the 2,6-positions as shown in structures **II** and **III**, respectively, of Chart 2. The 2,6-placement of these groups gives longer wavelengths of absorption if one compares values of λ_{max} for dyes **2c**−**4c** with dyes **2h**−**4h** (Table 1). Dyes **2a**−**4a** with three 4-(dimethylamino)phenyl substituents have absorption maxima closer to values of λ_{max} for **2h**−**4h** with 2,4-di-4-(dimethylamino)phenyl substituents relative to values of λ_{max} for **2c**−**4c** with 2,6-di-4-(dimethylamino)phenyl substituents. This observation suggests that resonance contributions from structure **II** are dominant in the chromophore of **2a**−**4a**. With one 4-(dimethylamino)-phenyl substituent, placement at the 2-position (dyes **2i**−**4i**) gives longer wavelengths of absorption than placement at the 4-position (dyes **2j**−**4j**).

Reduction Potentials of Dyes 2−4. One concern with respect to the use of mitochondrial-specific, cationic photosensitizers for PDT is toxicity derived from disrup-

Table 1. Selected Physical and Photophysical Properties for Chalcogenopyrylium Dyes **2**−**4**

dye	λ_{max},[a] nm	$E°$, V[b] (vs Fc/Fc$^+$)	$\Phi(^1O_2) \pm 2\sigma$
2a	594	−0.76	0.011 ± 0.002
2b	655	−0.71	
2c	675	−0.62	0.004 ± 0.001
2d	665	−0.67	0.025 ± 0.002
2h	627	−0.63	0.005 ± 0.001
2i	624	−0.45	0.004 ± 0.001
2j	592	−0.33	
3a	631	−0.70	0.040 ± 0.004
3b	669	−0.63	
3c	704	−0.55	0.015 ± 0.001
3d	695	−0.60	0.045 ± 0.002
3f	689	−0.55	
3g	723	−0.39	
3h	657	−0.55	0.015 ± 0.001
3i	654	−0.39	0.007 ± 0.001
3j	620	−0.31	0.005 ± 0.002[c]
4a	672	−0.62	0.018 ± 0.003
4b	703	−0.58	
4c	738	−0.49	0.007 ± 0.002
4d	727	−0.50	0.027 ± 0.002
4e	746	−0.45	0.006 ± 0.002
4f	729	−0.49	
4g	771	−0.35	
4h	689	−0.47	0.010 ± 0.001
4i	693	−0.33	0.004 ± 0.001
4j	653	−0.24	0.004 ± 0.001[c]

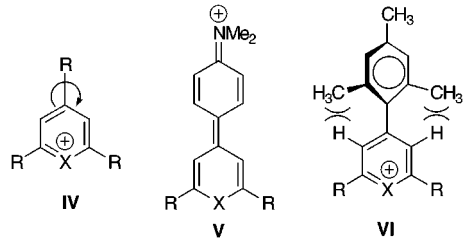
[a] In CH_2Cl_2. [b] In CH_2Cl_2 at a Pt disk electrode with a scan rate of 0.1 V s^{-1} with 0.2 M Bu_4NBF_4 as supporting electrolyte. [c] Reference 23.

tion of the redox cascade in the mitochondria. Typically, cationic dyes are good electron acceptors, and electron transfer within the mitochondria should be possible. The reduction potentials of dyes **2**−**4** were determined by cyclic voltammetry, and values of $E°$ for the cation/neutral radical couple are compiled in Table 1. As is observed with other chalcogenopyrylium dyes, replacing a heavier chalcogen atom with a lighter chalcogen atom in the ring leads to a cathodic (negative) shift in reduction potential for all substitution patterns shown in Table 1.[21] Electron-donating groups give cathodic shifts in reduction potentials (more negative values of $E°$), while electron-withdrawing groups give anodic shifts. The dye series **2**−**4** covers a 0.52-V range in values of $E°$ from −0.24 V for **4j** to −0.76 V for **2a** [vs ferrocene/ferricinium (Fc/Fc$^+$) at +0.40 V].

***n*-Octanol/Water Partition Coefficients for Dyes 2−4.** The dyes **2**−**4** all had similar values of log *P*, where *P* is the *n*-octanol/water partition coefficient. The range of values was 2.1−2.4 with the exception of dyes **3g** and **4g** with 3,5-di(trifluoromethyl)phenyl substituents at the 4-position where log *P* was 2.9 for both. These values can be compared to values of log *P* of 1.5 for Rh-123, 1.9 for AA1, and 2.4 for dye **1** (X = Te, Y = Se).

Quantum Yields for Singlet Oxygen Generation. Many of the cationic photosensitizers for PDT such as the Victoria blues,[2] methylene blues[3] and the related nile blues,[4] cyanine dyes,[5] rhodacyanine dyes,[6] and chalcogenopyrylium dyes **1**[7] generate singlet oxygen upon irradiation in aerated solvents. Singlet oxygen production upon irradiation is thought to be responsible for part if not all of the biological damage that is observed from PDT with these sensitizers both in vitro and in vivo. We have recently shown that the photodamage produced in vitro from dyes **2a**−**4a** is derived from the production of singlet oxygen.[16] The other

Chart 3



analogues **2**−**4** in this study would be expected to follow a similar mechanism. The quantum yields for singlet oxygen generation [$\Phi(^1O_2)$] for many of the dyes **2**−**4** were measured and are compiled in Table 1.

Several generalizations can be made with respect to the effects of substituents on values of $\Phi(^1O_2)$ for dyes **2**−**4**. For a given set of substituents, the selenopyrylium dye has a higher value of $\Phi(^1O_2)$ than the telluropyrylium dye, which in turn has a higher value of $\Phi(^1O_2)$ than the corresponding thiopyrylium dye. Presumably, the selenopyrylium nucleus is the best compromise for maximizing spin−orbit effects that promote singlet oxygen generation while minimizing geometry distortions as the larger chalcogens disrupt planarity in the π-framework.[16]

The excited state of chalcogenopyrylium dyes **2**−**4** can return to ground state via many processes including intersystem crossing to the triplet, which can then interact with ground-state (triplet) oxygen to produce singlet oxygen and ground-state dye. One process that returns excited state to ground state is the rotational release of energy by the substituents. Compounds bearing substituents that hinder free rotation around the C−C bond joining the substituents to the ring have higher values of $\Phi(^1O_2)$ than compounds with substituents that do not hinder rotation.

2,4,6-Triphenylselenopyrylium hexafluorophosphate has a value of $\Phi(^1O_2)$ of 0.003.[24] In the excited state, a phenyl substituent is free to rotate as shown illustrated in structure **IV** of Chart 3. The addition of a dimethylamino group on the phenyl ring produces higher values of $\Phi(^1O_2)$ as found in dye **3i** with a value of $\Phi(^1O_2)$ of 0.007 (Table 1) or dye **3j** with a value of $\Phi(^1O_2)$ of 0.005.[24] As shown in structure **V** of Chart 3, delocalization of the positive charge to nitrogen would create partial double-bond character to the C−C bond joining the substituent to the ring. The hindered rotation about the C−C bond increases the relative amount of intersystem crossing to the triplet and the value of $\Phi(^1O_2)$. Dyes **3c** and **3h** with two 4-(dimethylmino)phenyl substituents have higher values of $\Phi(^1O_2)$ of 0.015. Selenopyrylium dye **3a** bearing three 4-(dimethylamino)phenyl substituents has an even higher value of $\Phi(^1O_2)$ of 0.040 (Table 1).

Replacing the phenyl group of **3c** with a mesityl group in dye **3d** increases the value of $\Phi(^1O_2)$ to 0.045. The mesityl group also hinders rotation about the C−C bond joining the substituent to the ring through the steric interactions of the methyl groups as shown in structure **VI** of Chart 3. The steric hindrance to rotation leads to a 3-fold increase in $\Phi(^1O_2)$ for **3d** relative to **3c**.

The same trends are observed with thiopyrylium dyes **2** and telluropyrylium dyes **4**. However, both of these heterocyclic systems have lower values of $\Phi(^1O_2)$ relative to the corresponding selenopyrylium dye.

Although values of $\Phi(^1O_2)$ are small for dyes **2**−**4** relative to solution values of $\Phi(^1O_2)$ for other photosensitizers for PDT, the effects of rigidization in biological membranes on values of $\Phi(^1O_2)$ have not been determined.[25] Rotational degrees of freedom in rigid, planar molecules such as the porphyrins, methylene blues, and nile blues are few, and the photophysics of these sensitizers will be little affected when the sensitizers reside in biological membranes. If the dyes **2**−**4** were immobilized in a biological membrane, rotational degrees of freedom would be greatly reduced and effective values of $\Phi(^1O_2)$ would be higher than solution values. Several of the chalcogenopyrylium dyes **1** with solution values of $\Phi(^1O_2) < 0.01$ have performed as efficient photosensitizers in vitro.[2a,7a]

Biological Results and Discussion

In Vitro Clonogenic Assay. The toxicity of the individual chalcogenopyrylium dyes **2**−**4** will impact selection of sensitizer candidates for PDT. In particular, little is known about the relationship of the heteroatom in the chalcogenopyrylium dyes with the acute and chronic toxicities of these materials. As a first step in developing the comparative toxicity of the chalcogenopyrylium dyes, dyes **2**−**4** were evaluated for their inhibitory effects on the growth of several human carcinoma cell lines: colon carcinoma (CX-1), prostate carcinoma (PC-3), and breast carcinoma (MDA-435). In each case, the cells were incubated with one of the dyes **2**−**4** for 24 h and then in dye-free medium for 2 weeks. The number of colonies was measured by a crystal violet-staining method, and the results are expressed as the IC_{50} value and are compiled in Table 2 (Supporting Information). The magnitude of the IC_{50} value for each dye is a direct measure of toxicity.

For any particular substitution pattern at the 2-, 4-, and 6-positions, thiopyrylium dye **2**, selenopyrylium dye **3**, and telluropyrylium dye **4** all had similar IC_{50} values (within a factor of 3) toward any particular cell line as shown. The one exception to this generalization was the series **2i**−**4i** in which the IC_{50} values for thiopyrylium analogue **2i** indicated 3−9-fold greater toxicity than that found for the telluropyrylium analogue **4i** for all three cell lines. In general, however, the heteroatom had no predictable impact on toxicity in vitro.

The selection of substituents at the 2-, 4-, and 6-positions of dyes **2**−**4** has more of an impact on toxicity than the heteroatom. Substituent changes in the selenopyrylium dyes **3** and telluropyrylium dyes **4** gave a greater than 200-fold range of IC_{50} values among the cell lines of Table 2. In general, the 4-methylchalcogenopyrylium salts **3b** and **4b** displayed the least toxicity against all three cell lines relative to the other dyes, while the 2,6-di[4-(dimethylamino)phenyl]-4-phenylchalcogenopyrylium salts **2c**−**4c** displayed the most.

Electrochemical Reduction Potentials and in Vitro Toxicity. In the search for selective anticarcinoma agents, specific interactions of some structural feature in the drug with the particular carcinoma cell line can give greater specificity than toxicity derived from an intrinsic chemical property such as reduction potential or the *n*-octanol/water partition coefficient. There are only small differences in lipophilicity among the dyes **2**−**4** (with the exception of **3g** and **4g**). However, reduction potentials vary by 0.52 V among the dyes. With cationic dyes and other DLCs targeted to the mitochondria, electron transfer to the dye or DLC can disrupt mitochondrial processes and give cell death. Figure 1 shows a plot of IC_{50} values for dyes **2**−**4** against the CX-1 cell line as a function of reduction potential ($E°$). The lack of an apparent correlation suggests that differences in IC_{50} values can be attributed to specific drug−cell interactions in the clonogenic assays.

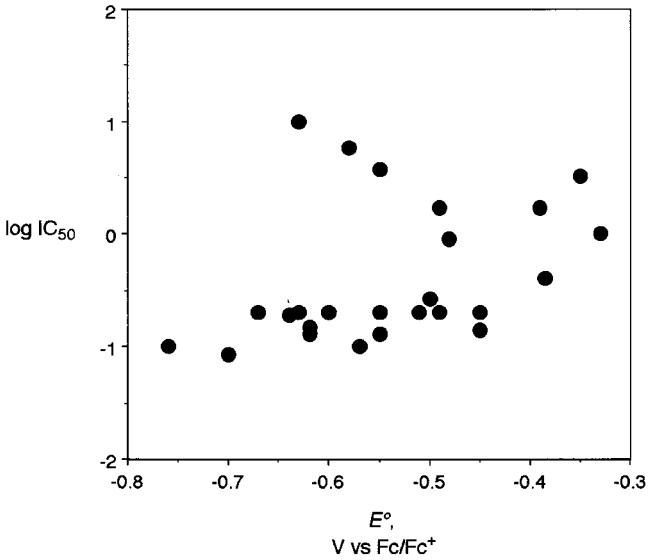


Figure 1. Plot of log IC_{50} values (μM in dye) for dyes **2**−**4** against CX-1 cells as determined by clonogenic assay as a function of the electrochemical reduction potential $E°$ (V vs Fc/Fc^+) for dyes **2**−**4**.

Initial in Vivo Toxicity Studies. The mitochondrial accumulation of cationic sensitizers for PDT or DLCs as selective anticarcinoma agents raises concerns that such materials will have high dark or inherent toxicity. In addition, cationic dyes are typically good electron acceptors and might be expected to interfere with electron transport at high concentrations. Nonlethal doses of 5−10 mg (13−26 μmol)/kg/day of Rh-123 have been administered for 10 consecutive days.[8c] Useful dosages of cationic sensitizers for use in PDT are typically much smaller and are delivered as a single dosage in the 5−10 μmol/kg range.[1,22]

With selenopyrylium dyes **3** and telluropyrylium dyes **4**, the toxicity associated with the heteroatom is an added concern. Selenium is an essential trace element and has been the subject of numerous toxicity studies.[26,27] One is tempted to assume that since tellurium is below selenium in the periodic table, it must be more metallic and consequently more toxic. Toxicity with respect to tellurium metal, which is in commercial use in the semiconductor and reprographic industries, has been reviewed, and in general, tellurium has been found to be less toxic than selenium.[26,27] Daily ingestion of tellurium from air, food, and fluids is estimated to be as high as 0.6 mg (4−5 μmol)/day in some areas for a 70-kg adult.[26] Other tellurium-containing compounds have emerged in recent years as potential drugs and have shown acceptable toxicity in animal studies.[28] Ammonium trichloro(dioxoethylene-*O*,*O*′)tellurate (AS-101), an inorganic tellurate complex, was shown to have

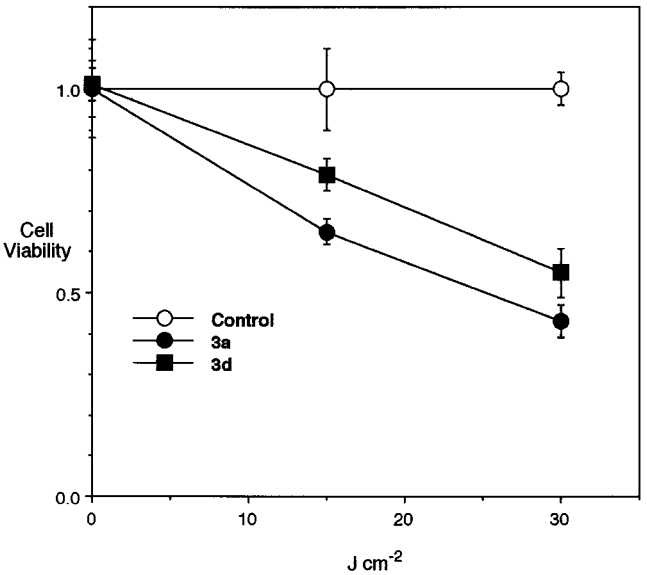


Figure 2. Effects of selenopyrylium dye **3a** or **3d** photosensitization on the cell viability of cultured Colo-26 tumor cells. Details of experimental conditions are described in the Experimental Section. Data are expressed as the fraction of viable cells compared to untreated cells (○) and for cells treated with 1 μM solutions of dye **3a** (●) or dye **3d** (■) and light. Each datum point represents the mean percent viable cells calculated from at least 3 separate experiments; bars are the standard deviation.

both antitumor and immunomodulatory activity in vivo.[28]

Dyes **2**−**4** were evaluated for dark toxicity in BALB/c mice given a single tail-vein injection of dye dissolved in 1% Tween 80 in saline. Generally, groups of five mice were tested with escalating concentrations of dye and followed for 60 days after injection. The maximum dye dose was determined by its solubility in the 1% Tween 80/saline vehicle. For selenopyrylium dye **3h**, no toxicity was observed with 7.7 mg (19 μmol) of dye/kg of animal. For dyes **2a**−**4a**, no toxicity was observed following injection of 4.0 mg (8.3 μmol) of thiopyrylium dye **2a**/kg, 20 mg (38 μmol) of selenopyrylium dye **3a**/kg, and 7.0 mg (12 μmol) of telluropyrylium dye **4a**/kg. However, a group of three animals given a dosage of 27 mg (50.3 μmol) of selenopyrylium dye **3a**/kg had no surviving animals after 48 h. For dyes **2d**−**4d**, no toxicity was observed following injection of 4.4 mg (9.2 μmol) of thiopyrylium dye **2d**/kg (10 animals), 8.6 mg (16 μmol) of selenopyrylium dye **3d**/kg, and 11.0 mg (19 μmol) of telluropyrylium dye **4d**/kg. For telluropyrylium dye **4g**, no toxicity was observed at 5.0 mg (7.2 μmol) of dye/kg.

Photodynamic Therapy against Colo-26 Cells in Culture. Dyes **3a** and **3d** had the two highest quantum yields for singlet oxygen generation (0.040 and 0.045, respectively) in the series of dyes **2**−**4** (Table 1) and were not dark toxic in vivo at 10 μmol of dye/kg, a typical therapeutic dosage for PDT. The dyes were tested as PDT photosensitizers in vitro against murine Colo-26 cells. Cell cultures incubated for 2 h with 1 μM concentrations of either dye **3a** or **3d** displayed essentially no dark toxicity associated with the dye. As shown in Figure 2, irradiation of dye-treated cells resulted in a light-dose-dependent reduction of cell viability that was significantly greater than that observed for irradiation of nontreated cells ($P < 0.001$ at 30 J cm^{-2}).

Summary and Conclusions

A series of thiopyrylium, selenopyrylium, and telluropyrylium dyes (**2**−**4**, respectively) with diverse substituents was prepared. The absorption spectra and quantum yields for singlet oxygen generation of these dyes suggested that the dyes would have utility as sensitizers for PDT. Dyes **3a** and **3d** with the highest quantum yields for singlet oxygen generation were found to be photosensitizers in vitro against Colo-26 cells. The dark toxicity of the dyes **2**−**4** was evaluated in clonogenic assays of human carcinoma cell lines and normal monkey epithelial cells in order to compare the relative toxicity of thiopyrylium, selenopyrylium, and telluropyrylium analogues with identical carbon π-frameworks. Importantly, the presence of a sulfur, selenium, or tellurium heteroatom in the molecules had no predictable impact on the dark toxicity of any particular dye set. Substituents at the 2-, 4-, and 6-positions of the dye had a much greater impact on cytotoxicity. The IC_{50} values determined in the clonogenic assays did not correlate with chemical properties in the dye molecules such as reduction potential or lipophilicity. Initial in vivo toxicity studies showed little toxicity at 7.2−38 μmol/kg dosages in BALB/c mice.

Experimental Section

General Methods. Solvents and reagents were used as received from Sigma-Aldrich Chemical Co. (St. Louis, MO) unless otherwise noted. Cell culture media was purchased from GIBCO (Grand Island, NY). Fetal bovine serum (FBS) was obtained from Atlanta Biologicals (Atlanta, GA). Concentration in vacuo was performed on a Büchi rotary evaporator. NMR spectra were recorded on a Varian Gemini-300, Inova 400, or Inova 500 instrument with residual solvent signal as internal standard: $CDCl_3$ (δ 7.26 for proton, δ 77.0 for carbon). Infrared spectra were recorded on a Perkin-Elmer FT-IR instrument. UV−visible near-IR spectra were recorded on a Perkin-Elmer Lambda 12 spectrophotometer or on a Sequential DX17 MV stopped-flow spectrometer (Applied Photophysics, Leatherhead, U.K.). Both were equipped with a circulating constant-temperature bath for the sample chambers. Elemental analyses were conducted by Atlantic Microanalytical, Inc. Dyes were analyzed as the hexafluorophosphate salts since the chloride salts were hygroscopic.

2,6-Di[4-(dimethylamino)phenyl]-4-methylthiopyrylium Chloride (2b). Methylmagnesium bromide (1.4 M in THF, 1.0 mL) was added dropwise to a solution of thiopyranone **5a** (0.150 g, 0.428 mmol) in THF (10 mL) under an argon atmosphere. The resulting mixture was heated at reflux for 3 h, then cooled. The reaction mixture was concentrated. The residue was dissolved in acetic acid (10 mL) and added dropwise to 10% HPF_6 (10 mL) at 0 °C. The product was collected by filtration and washed with water (4 × 10 mL) and ether (4 × 10 mL). The solid was redissolved in a small amount of acetone, added to H_2O (200 mL), and recollected by filtration. The crude product was dissolved in 10 mL of methanol and 0.3 g of Amberlite IRA-400 Cl ion-exchange resin was added. The resulting mixture was stirred 0.5 h and the exchange resin was removed via filtration. The process was repeated with two additional 0.3-g aliquots of the ion-exchange resin. The product was recrystallized from CH_2Cl_2/ether to give 0.122 g (74%) of **2b**: mp 228−231 °C; ^{1}H NMR (CD_2Cl_2) δ 7.83 (m, 6 H), 6.85 ("doublet" AA′BB′, 4 H, J = 8.7 Hz), 3.16 (s, 12 H), 2.70 (s, 3 H); ^{13}C NMR (CD_2Cl_2) δ 161.1, 155.5, 151.8, 135.5, 129.5, 127.3, 127.0, 125.7, 120.1, 118.1, 110.2, 37.6; λ_{max} (CH_2Cl_2) 655 nm [ϵ (5.5 ± 0.2) × 10^4 M^{-1} cm^{-1}]. Anal. as the hexafluorophosphate salt ($C_{22}H_{25}F_6N_2PS$) C, H, N.

2,6-Di[4-(dimethylamino)phenyl]-4-methylselenopyrylium Chloride (3b). Methylmagnesium bromide (1.4 M in THF, 1.0 mL) was added dropwise to a solution of selenopy-

ranone **5a** (0.150 g, 0.378 mmol) in THF (10 mL) under an argon atmosphere. The resulting mixture was treated as described above for **2b**. The product was recrystallized from CH_2Cl_2/ether to give 0.111 g (68%) of **3b**: mp 229−231 °C; ^{1}H NMR (CD_2Cl_2) δ 7.77 (m, 6 H), 6.84 ("doublet" AA′BB′, 4 H, J = 9.0 Hz), 3.15 (s, 12 H), 2.63 (s, 3 H); λ_{max} (CH_2Cl_2) 669 nm [ϵ (7.5 ± 0.2) × 10^4 M^{-1} cm^{-1}]. Anal. as the hexafluorophosphate salt ($C_{22}H_{25}F_6N_2PSe$) C, H, N.

2,6-Di[4-(dimethylamino)phenyl]-4-methyltelluropyrylium Chloride (4b). Methylmagnesium bromide (1.4 M in THF, 1.0 mL) was added dropwise to a solution of telluropyranone **5a** (0.100 g, 0.224 mmol) in THF (10 mL) under an argon atmosphere. The resulting mixture was treated as described above for **2b**. The product was recrystallized from CH_2Cl_2/ether to give 0.075 g (70%) of **4b**: mp 238−240 °C; ^{1}H NMR (CD_2Cl_2) δ 7.74 (s, 2 H), 7.66 ("doublet" AA′BB′, 4 H, J = 9.0 Hz), 6.81 ("doublet" AA′BB′, 4 H, J = 9.0 Hz), 3.13 (s, 12 H), 2.53 (s, 3 H); ^{13}C NMR (CD_2Cl_2) δ 171.9, 158.6, 152.4, 127.4, 126.2, 124.7, 110.4, 37.6, 26.4; λ_{max} (CH_2Cl_2) 703 nm [ϵ (7.1 ± 0.2) × 10^4 M^{-1} cm^{-1}]. Anal. as the hexafluorophosphate salt ($C_{22}H_{25}F_6N_2PTe$) C, H, N.

2,6-Di[4-(dimethylamino)phenyl]-4-phenylthiopyrylium Chloride (2c). A mixture of bromobenzene (0.060 mL, 0.57 mmol) and magnesium turnings (0.027 g, 1.1 mg-at) was heated at reflux in THF (10 mL) for 3 h. A solution of thiopyranone **5a** (0.100 g, 0.285 mmol) in 5 mL of anhydrous THF was added dropwise to the reaction mixture and the resulting solution was heated at reflux for an additional hour. The reaction mixture was concentrated. The residue was dissolved in acetic acid (10 mL) and added dropwise to 10% HPF_6 (10 mL) at 0 °C. The product was collected by filtration and washed with water (4 × 10 mL) and ether (4 × 10 mL). The solid was redissolved in a small amount of acetone, added to H_2O (200 mL), and recollected by filtration. The crude product was dissolved in 10 mL of methanol and 0.3 g of Amberlite IRA-400 Cl ion-exchange resin was added. The resulting mixture was stirred 0.5 h and the exchange resin was removed via filtration. The process was repeated with two additional 0.3-g aliquots of the ion-exchange resin. The product was recrystallized from CH_3CN to give 0.102 g (80%) of **2c**: mp 238−240 °C; ^{1}H NMR (CD_2Cl_2) δ 8.15 (s, 2 H), 7.86 (m, 6 H), 7.66 (m, 3 H), 6.88 ("doublet" AA′BB′, 4 H, J = 9.2 Hz), 3.18 (s, 12 H); ^{13}C NMR (CD_2Cl_2) δ 161.1, 155.5, 151.8, 135.5, 129.5, 127.3, 127.0, 125.7, 120.1, 118.1, 110.2, 37.6; λ_{max} (CH_2Cl_2) 675 nm [ϵ (4.8 ± 0.1) × 10^4 M^{-1} cm^{-1}]. Anal. as the hexafluorophosphate salt ($C_{27}H_{27}F_6N_2PS$) C, H, N.

2,6-Di[4-(dimethylamino)phenyl]-4-phenylselenopyrylium Chloride (3c). A mixture of bromobenzene (0.060 mL, 0.57 mmol) and magnesium turnings (0.036 g, 1.5 mg-at) was heated at reflux in THF (10 mL) for 3 h. A solution of selenopyranone **5a** (0.150 g, 0.378 mmol) in 5 mL of anhydrous THF was added dropwise to the reaction mixture and the resulting solution was treated as described above for **2c**. The product was recrystallized from CH_3CN to give 0.095 g (51%) of **3c**: mp >260 °C; ^{1}H NMR (CD_2Cl_2) δ 8.03 (s, 2 H), 7.81 (m, 5 H), 7.64 ("doublet" AA′BB′, 4 H, J = 9.2 Hz), 6.85 ("doublet" AA′BB′, 4 H, J = 9.2 Hz), 3.16 (s, 12 H); ^{13}C NMR (CD_2Cl_2) δ 167.6, 155.2, 151.8, 136.9, 128.9, 127.2, 127.1, 125.7, 120.4, 119.9, 110.3, 37.6; λ_{max} (CH_2Cl_2) 704 nm [ϵ (6.8 ± 0.2) × 10^4 M^{-1} cm^{-1}]. Anal. as the hexafluorophosphate salt ($C_{27}H_{27}F_6N_2PSe$) C, H, N.

2,6-Di[4-(dimethylamino)phenyl]-4-phenyltelluropyrylium Chloride (4c). A mixture of bromobenzene (0.14 g, 0.89 mmol) and magnesium turnings (0.044 g, 1.8 mg-at) was heated at reflux in THF (10 mL) for 3 h. A solution of telluropyranone **5a** (0.200 g, 0.448 mmol) in 5 mL of anhydrous THF was added dropwise to the reaction mixture and the resulting solution was treated as described above for **2c**. The product was recrystallized from CH_3CN to give 0.144 g (59%) of **4c**: mp 250−252 °C; ^{1}H NMR (CD_2Cl_2) δ 7.98 (s, 2 H), 7.78 (m, 3 H), 7.69 ("doublet" AA′BB′, 4 H, J = 9.2 Hz), 7.62 (m, 2 H), 6.83 ("doublet" AA′BB′, 4 H, J = 9.2 Hz), 3.14 (s, 12 H); ^{13}C NMR (CD_2Cl_2) δ 171.5, 157.6, 152.2, 140.0, 128.3, 127.6, 127.2, 125.4, 125.3, 124.9, 110.5, 37.7; λ_{max} (CH_2Cl_2) 738 nm [ϵ (5.79 ± 0.02) × 10^4 M^{-1} cm^{-1}]. Anal. as the hexafluorophosphate salt ($C_{27}H_{27}F_6N_2PTe$) C, H, N.

2,6-Di[4-(dimethylamino)phenyl]-4-(2,4,6-trimethylphenyl)thiopyrylium Chloride (2d). A mixture of bromomesitylene (0.24 g, 0.180 mL, 1.2 mmol) and magnesium turnings (0.060 g, 2.5 mg-at) was heated at reflux in anhydrous THF (50 mL) for 3 h. A solution of thiopyranone **5a** (0.20 g, 0.62 mmol) in 5 mL of anhydrous THF was added dropwise to the reaction mixture and the resulting solution was heated at reflux for an additional hour. The reaction mixture was concentrated. The residue was dissolved in acetic acid (10 mL) and added dropwise to 10% HPF_6 (10 mL) at 0 °C. The product was collected by filtration and washed with water (4 × 10 mL) and ether (4 × 10 mL). The solid was redissolved in a small amount of acetone, added to H_2O (200 mL), and recollected by filtration. The crude product was dissolved in 10 mL of methanol and 0.3 g of Amberlite IRA-400 Cl ion-exchange resin was added. The resulting mixture was stirred 0.5 h and the exchange resin was removed via filtration. The process was repeated with two additional 0.3-g aliquots of the ion-exchange resin. The product was recrystallized from CH_3CN to give 0.24 g (66%) of **2d**: mp 209−211 °C; ^{1}H NMR (CD_2Cl_2) δ 7.85 ("doublet" AA′BB′, 4 H, J = 9.2 Hz), 7.69 (s, 2 H), 7.03 (s, 2 H), 6.87 ("doublet" AA′BB′, 4 H, J = 9.2 Hz), 3.17 (s, 12 H), 2.36 (s, 3 H), 2.17 (s, 6 H); ^{13}C NMR (CD_2Cl_2) δ 164.5, 160.8, 154.9, 140.0, 136.6, 134.8, 130.0, 129.5, 125.0, 120.7, 113.3, 40.6, 21.4, 20.1; λ_{max} (CH_2Cl_2) 665 nm [ϵ (7.2 ± 0.1) × 10^4 M^{-1} cm^{-1}]. Anal. as the hexafluorophosphate salt ($C_{30}H_{33}F_6N_2PS-H_2O$) C, H, N.

2,6-Di[4-(dimethylamino)phenyl]-4-(2,4,6-trimethylphenyl)selenopyrylium Chloride (3d). A mixture of bromomesitylene (0.33 g, 0.29 mL, 1.5 mmol) and magnesium turnings (0.080 g, 3.0 mg-at) was heated at reflux in anhydrous THF (50 mL) for 5 h. A solution of selenopyranone **5a** (0.33 g, 0.83 mmol) in 30 mL of anhydrous THF was added dropwise to the reaction mixture and the resulting solution was treated as described above for **2d**. The product was recrystallized from CH_3CN to give 0.24 g (45%) of **3d**: mp 209−211 °C; ^{1}H NMR (CD_2Cl_2) δ 7.87 ("doublet" AA′BB′, 4 H, J = 9.2 Hz), 7.57 (s, 2 H), 7.02 (s, 2 H), 6.85 ("doublet" AA′BB′, 4 H, J = 9.2 Hz), 3.15 (s, 12 H), 2.36 (s, 3 H), 2.15 (s, 6 H); ^{13}C NMR (CD_2Cl_2) δ 167.0, 156.1, 150.3, 134.9, 133.2, 129.9, 125.4, 124.65, 120.35, 118.5, 108.7, 35.9, 16.65, 16.1; λ_{max} (CH_2Cl_2) 695 nm [ϵ (6.3 ± 0.1) × 10^4 M^{-1} cm^{-1}]. Anal. as the hexafluorophosphate salt ($C_{30}H_{33}F_6N_2PSe$) C, H, N.

2,6-Di[4-(dimethylamino)phenyl]-4-(2,4,6-trimethylphenyl)telluropyrylium Chloride (4d). A mixture of bromomesitylene (0.29 g, 0.22 mL, 1.0 mmol) and magnesium turnings (0.060 g, 2.5 mg-at) was heated at reflux in anhydrous THF (50 mL) for 4 h. A solution of telluropyranone **5a** (0.33 g, 0.74 mmol) in 10 mL of anhydrous THF was added dropwise to the reaction mixture and the resulting solution was treated as described above for **2d**. The product was recrystallized from CH_3CN to give 0.38 g (75%) of **4d**: mp 238−240 °C; ^{1}H NMR (CD_2Cl_2) δ 7.67 ("doublet" AA′BB′, 4 H, J = 9 Hz), 7.54 (s, 2 H), 7.01 (s, 2 H), 6.78 ("doublet" AA′BB′, 4 H, J = 9 Hz), 3.12 (s, 12 H), 2.36 (s, 3 H), 2.13 (s, 6 H); ^{13}C NMR (CD_2Cl_2) δ 169.9, 156.9, 150.4, 135.2, 129.6, 125.8, 124.6, 123.6, 123.1, 108.8, 35.95, 16.4, 16.0; λ_{max} (CH_2Cl_2) 727 nm [ϵ (6.9 ± 0.1) × 10^4 M^{-1} cm^{-1}]. Anal. as the hexafluorophosphate salt ($C_{30}H_{33}F_6N_2PTe$) C, H, N.

2,6-Di[4-(dimethylamino)phenyl]-4-(2-thienyl)telluropyrylium Chloride (4e). A mixture of 2-bromothiophene (0.274 g, 1.68 mmol) and magnesium turnings (0.082 g) was heated at reflux in anhydrous THF (30 mL) under argon atmosphere for 3 h. A solution of telluropyranone **5a** (0.300 g, 0.673 mmol) in THF (20 mL) was added dropwise to the reaction mixture and the resulting mixture was heated at reflux for an additional 1 h. The reaction mixture was concentrated. The residue was dissolved in acetic acid (10 mL) and added dropwise to 10% HPF_6 (10 mL) at 0 °C. The product was collected by filtration and washed with water (4 × 10 mL) and ether (4 × 10 mL). The solid was redissolved in a small amount of acetone, added to H_2O (200 mL), and recollected by

filtration. The crude product was dissolved in 10 mL of methanol and 0.3 g of Amberlite IRA-400 Cl ion-exchange resin was added. The resulting mixture was stirred 0.5 h and the exchange resin was removed via filtration. The process was repeated with two additional 0.3-g aliquots of the ion-exchange resin. The product was recrystallized from CH_3CN to give 0.248 g (67%) of **4e**: mp 244−246 °C; ^{1}H NMR (CD_2Cl_2) δ 8.10 (s, 2 H), 8.03 ("doublet" AA′BB′, 4 H, J = 9 Hz), 7.82 ("doublet" AA′BB′, 4 H, J = 9 Hz), 7.65 ("doublet" AA′BB′, 4 H, J = 8.7 Hz), 7.31 (t, 1 H, J = 9 Hz), 6.81 ("doublet" AA′BB′, 4 H, J = 8.7 Hz), 3.13 (s, 12 H); ^{13}C NMR (CD_2Cl_2) δ 173.9, 156.8, 153.0, 147.8, 134.5, 133.5, 132.8, 132.5, 129.9, 126.1, 115.3, 42.7; λ_{max} (CH_2Cl_2) 746 nm [ϵ (9.4 ± 0.7) × 10^4 M^{-1} cm^{-1}]. Anal. as the hexafluorophosphate salt ($C_{25}H_{25}F_6N_2PSTe$·1/4CH_3CN) C, H, N.

2,6-Di[4-(dimethylamino)phenyl]-4-(3,5-dimethylphenyl)selenopyrylium Chloride (3f). A mixture of bromo-3,5-dimethylbenzene (0.37 g, 2.0 mmol) and magnesium turnings (0.10 g, 4.4 mg-at) was heated at reflux in anhydrous THF (50 mL) for 5 h. A solution of selenopyranone **5a** (0.33 g, 0.83 mmol) in 30 mL of anhydrous THF was added dropwise to the reaction mixture and the resulting solution was heated at reflux for an additional hour. The reaction mixture was concentrated. The residue was dissolved in acetic acid (10 mL) and added dropwise to 10% HPF_6 (10 mL) at 0 °C. The product was collected by filtration and washed with water (4 × 10 mL) and ether (4 × 10 mL). The solid was redissolved in a small amount of acetone, added to H_2O (200 mL), and recollected by filtration. The crude product was dissolved in 10 mL of methanol and 0.3 g of Amberlite IRA-400 Cl ion-exchange resin was added. The resulting mixture was stirred 0.5 h and the exchange resin was removed via filtration. The process was repeated with two additional 0.3-g aliquots of the ion-exchange resin. The product was recrystallized from CH_3CN to give 0.43 g (62%) of **3f**: mp 209−211 °C; ^{1}H NMR (CD_2Cl_2) δ 8.02 (s, 2 H), 7.87 ("doublet" AA′BB′, 4 H, J = 9.2 Hz), 7.48 (s, 2 H), 7.27 (s, 1 H), 6.81 ("doublet" AA′BB′, 4 H, J = 9 Hz), 3.09 (s, 12 H), 2.42 (s, 6 H); λ_{max} (CH_2Cl_2) 689 nm [ϵ (6.3 ± 0.1) × 10^4 M^{-1} cm^{-1}]. Anal. as the hexafluorophosphate salt ($C_{29}H_{31}F_6N_2$-PSe) C, H, N.

2,6-Di[4-(dimethylamino)phenyl]-4-(3,5-dimethylphenyl)telluropyrylium Chloride (4f). A mixture of bromo-3,5-dimethylbenzene (0.28 g, 1.5 mmol) and magnesium turnings (0.070 g, 2.9 mg-at) was heated at reflux in anhydrous THF (50 mL) for 5 h. A solution of telluropyranone **5a** (0.33 g, 0.74 mmol) in 30 mL of anhydrous THF was added dropwise to the reaction mixture and the resulting solution was treated as described above for **3f**. The product was recrystallized from CH_3CN to give 0.34 g (67%) of **4f**: mp 209−211 °C; ^{1}H NMR (CD_2Cl_2) δ 8.01 (s, 2 H), 7.73 ("doublet" AA′BB′, 4 H, J = 9.2 Hz), 7.48 (s, 2 H), 7.27 (s, 1 H), 6.81 ("doublet" AA′BB′, 4 H, J = 9 Hz), 3.09 (s, 12 H), 2.42 (s, 6 H); λ_{max} (CH_2Cl_2) 729 nm [ϵ (6.3 ± 0.1) × 10^4 M^{-1} cm^{-1}]. Anal. as the hexafluorophosphate salt ($C_{29}H_{31}F_6N_2PTe$) C, H, N.

2,6-Di[4-(dimethylamino)phenyl]-4-[3,5-di(trifluoromethyl)phenyl]selenopyrylium Chloride (3g). A mixture of bromo-3,5-di(trifluoromethyl)benzene (0.50 g, 1.7 mmol) and magnesium turnings (0.080 g, 3.3 mg-at) was heated at reflux in anhydrous THF (20 mL) for 5 h. A solution of selenopyranone **5a** (0.33 g, 0.83 mmol) in 30 mL of anhydrous THF was added dropwise to the reaction mixture and the resulting solution was heated at reflux for an additional hour. The reaction mixture was concentrated. The residue was dissolved in acetic acid (10 mL) and added dropwise to 10% HPF_6 (10 mL) at 0 °C. The product was collected by filtration and washed with water (4 × 10 mL) and ether (4 × 10 mL). The solid was redissolved in a small amount of acetone, added to H_2O (200 mL), and recollected by filtration. The crude product was dissolved in 10 mL of methanol and 0.3 g of Amberlite IRA-400 Cl ion-exchange resin was added. The resulting mixture was stirred 0.5 h and the exchange resin was removed via filtration. The process was repeated with two additional 0.3-g aliquots of the ion-exchange resin. The product was recrystallized from CH_3CN to give 0.45 g (74%) of **3g**: mp 249−250 °C; ^{1}H NMR (CD_2Cl_2) δ 8.39 (s, 2 H), 8.24 (s, 1 H), 8.01 (s, 2 H), 7.85 ("doublet" AA′BB′, 4 H, J = 9.2 Hz), 6.85 ("doublet" AA′BB′, 4 H, J = 9 Hz), 3.13 (s, 12 H); ^{13}C NMR (CD_2Cl_2) δ 165.7, 149.7, 149.3, 137.0, 126.5 (q), 124.8, 123.9, 119.1, 118.0, 117.8, 116.3, 107.7, 34.5; λ_{max} (CH_2Cl_2) 723 nm [ϵ (4.5 ± 0.1) × 10^4 M^{-1} cm^{-1}]. Anal. as the hexafluorophosphate salt ($C_{29}H_{25}F_{12}N_2PSe$) C, H, N.

2,6-Di[4-(dimethylamino)phenyl]-4-[3,5-di(trifluoromethyl)phenyl]telluropyrylium Chloride (4g). A mixture of bromo-3,5-di(trifluoromethyl)benzene (0.50 g, 1.7 mmol) and magnesium turnings (0.080 g, 3.3 mg-at) was heated at reflux in anhydrous THF (20 mL) for 5 h. A solution of telluropyranone **5a** (0.33 g, 0.83 mmol) in 30 mL of anhydrous THF was added dropwise to the reaction mixture and the resulting solution was treated as described above for **3g**. The product was recrystallized from CH_3CN to give 0.30 g (51%) of **4 g**: mp ≥ 240 °C; ^{1}H NMR (CD_2Cl_2) δ 8.19 (s, 2 H), 8.18 (s, 1 H), 7.83 (s, 2 H), 7.75 ("doublet" AA′BB′, 4 H, J = 9.2 Hz), 6.90 ("doublet" AA′BB′, 4 H, J = 9 Hz), 3.20 (s, 12 H); ^{13}C NMR (CD_2Cl_2) δ 169.6, 151.3, 150.7, 140.3, 128.4 (q), 127.6, 123.3, 121.7, 120.3, 119.2, 116.7, 108.7, 35.8; λ_{max} (CH_2Cl_2) 771 nm [ϵ (7.6 ± 0.1) × 10^4 M^{-1} cm^{-1}]. Anal. as the hexafluorophosphate salt ($C_{29}H_{25}F_{12}N_2PTe$) C, H, N.

Preparation of 1-[4-(Dimethylamino)phenyl]-5-phenyl-1,4-pentadiyn-3-one (6b). Butyllithium (40 mL of a 2.5 M solution in hexanes) was added to a solution of 4-(dimethylamino)phenylacetylene (15.8 g, 0.100 mol) in THF (200 mL) at −78 °C. The resulting solution was stirred for 1 h at −78 °C. Phenylpropargyl aldehyde (11.8 g, 0.100 mol) was added and the resulting mixture was stirred for 3 h. Brine (100 mL) was added and the product was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated. The residue was purified via column chromatography on SiO_2 eluted with CH_2Cl_2/hexanes to give 1-[4-(dimethylamino)phenyl]-5-phenyl-1,4-pentadiyn-3-ol (19.3 g, 70%) as an orange solid: mp 116−118 °C; ^{1}H NMR ($CDCl_3$) δ 7.50 (m, 2 H), 7.34 (m, 5 H), 6.62 ("doublet" AA′BB′, 2 H, J = 9 Hz), 5.57 (d, 1 H, J = 6 Hz), 2.97 (s, 6 H), 2.37 (d, 1 H, J = 6 Hz); ^{13}C NMR ($CDCl_3$) δ 150.4, 133.0, 131.9, 128.7, 128.2, 122.1, 111.7, 108.5, 86.5, 85.9, 84.1, 83.9, 53.3, 40.1; IR (KBr) 3262 (s), 2923 (s), 2224 (s) cm^{-1}; FDMS m/z 277 ($C_{19}H_{19}NO$, 277). Anal. Calcd for $C_{19}H_{19}NO$: C, 82.29; H, 6.22; N, 5.09. Found: C, 82.66; H, 6.14; N, 5.11.

1-[4-(*N*,*N*-Dimethylamino)phenyl]-5-phenyl-1,4-pentadiyn-3-ol (9.60 g, 34.9 mmol) was dissolved in CH_2Cl_2 (200 mL). Manganese dioxide (15.2 g, 0.175 mol) was added and the resulting mixture was stirred at ambient temperature for 1 h. The reaction mixture was filtered through Celite and the filtrate was concentrated to give 9.12 g (95%) of diynone **6b**: mp 133−135 °C; ^{1}H NMR ($CDCl_3$) δ 7.63 (m, 2 H), 7.54 (m, 2 H), 7.39 (m, 3 H), 6.63 (dd, 2 H), 3.04 (s, 6 H); ^{13}C NMR ($CDCl_3$) δ 152.1, 135.6, 133.1, 130.8, 128.6, 119.9, 111.5, 104.6, 97.1, 90.1, 89.5, 39.9; IR (KBr) 2904 (b), 2145 (s), 1598 (s) cm^{-1}; FDMS m/z 275 ($C_{19}H_{19}NO$, 275). Anal. Calcd for $C_{19}H_{17}NO$: C, 83.49; H, 5.53; N, 5.13. Found: C, 83.31; H, 5.46; N, 5.10.

Δ-4*H*-2-[4-(Dimethylamino)phenyl]-6-phenylthiopyran-4-one [5b (X = S)]. A mixture of sulfur (0.282 g, 8.78 mmol), sodium borohydride (0.415 g, 11.0 mmol), and 0.25 M sodium ethoxide in ethanol (75 mL) was heated at reflux for 3 h until the reaction turned clear. 1-[4-(Dimethylamino)phenyl]-5-phenyl-1,4-pentadiyn-3-one (**6b**) (2.00 g, 7.32 mmol) was dissolved in THF (10 mL) and the resulting solution was added to 0.25 M sodium ethoxide in ethanol (10 mL) and stirred for 1 h until the diynone was converted to a mixture of enol ethers. The solution of enol ethers was added to the reaction mixture and stirring was continued for 1 h. Water (200 mL) was added and the product was extracted with CH_2Cl_2 (4 × 100 mL). The combined organic extracts were dried over $MgSO_4$, filtered through Celite, and concentrated. The residue was purified via column chromatography on SiO_2 eluted with CH_2Cl_2 to give 1.65 g (73%) of thiopyranone **5b** as a brown solid: mp 154−155 °C; ^{1}H NMR ($CDCl_3$) δ 7.66 (m, 2 H), 7.56 (m, 2H) 7.50 (m, 3H), 6.75 ("doublet" AA′BB′, 2 H, J = 9 Hz), 3.04 (s, 6 H); ^{13}C NMR ($CDCl_3$) δ 182.6, 153.7, 152.5, 151.9, 136.3, 130.4,

129.2, 127.6, 126.8, 126.7, 123.5, 122.6, 111.9, 40.0; IR (KBr) 3447, 3005, 1714 cm^{-1}; EIMS *m/z* 307 ($C_{19}H_{17}NOS$). Anal. ($C_{19}H_{17}NOS$) C, H, N.

Δ-4*H*-2-[4-(Dimethylamino)phenyl]-6-phenylselenopyran-4-one [5b (X = Se)]. A mixture of selenium (0.693 g, 8.78 mmol), sodium borohydride (0.415 g, 11.0 mmol), and 0.25 M sodium ethoxide in ethanol (75 mL) was heated at reflux for 3 h until the reaction turned clear. 1-[4-(Dimethylamino)phenyl]-5-phenyl-1,4-pentadiyn-3-one (**6b**) (2.00 g, 7.32 mmol) was dissolved in THF (10 mL) and the resulting solution was added to 0.25 M sodium ethoxide in ethanol (10 mL) and stirred for 1 h until the diynone was converted to a mixture of enol ethers. The solution of enol ethers was added to the reaction mixture and stirring was continued for 1 h. Water (200 mL) was added and the product was extracted with CH_2Cl_2 (4 × 100 mL). The combined organic extracts were dried over $MgSO_4$, filtered through Celite, and concentrated. The residue was purified via column chromatography on SiO_2 eluted with CH_2Cl_2 to give 1.77 g (68%) of selenopyranone **5b** as a brown solid: mp 155−156 °C; ^{1}H NMR ($CDCl_3$) δ 7.59−7.47 (m, 7 H), 7.25 (s, 2 H), 6.73 ("doublet" AA′BB′, 2 H, J = 9 Hz), 3.03 (s, 6 H); ^{13}C NMR ($CDCl_3$) δ 184.8, 156.2, 154.7, 152.0, 138.0, 130.4, 129.3, 128.0, 127.8, 126.8, 124.6, 124.5, 112.1, 40.1; IR (KBr) 3420, 2895, 1596 cm^{-1}; EIMS *m/z* 355 ($C_{19}H_{17}NO^{80}Se$). Anal. ($C_{19}H_{17}NOSe$) C, H, N.

Δ-4*H*-2-[4-(Dimethylamino)phenyl]-6-phenyltelluropyran-4-one [5b (X = Te)]. A mixture of tellurium granules (1.12 g, 8.78 mmol), sodium borohydride (0.415 g, 11.0 mmol), and 0.25 M sodium ethoxide in ethanol (75 mL) was heated at reflux for several hours until the reaction turned clear. 1-[4-(Dimethylamino)phenyl]-5-phenyl-1,4-pentadiyn-3-one (**6b**) (2.00 g, 7.32 mmol) was dissolved in THF (10 mL) and the resulting solution was added to 0.25 M sodium ethoxide in ethanol (10 mL). The resulting mixture was stirred for several hours until the diynone was converted to the enol ethers. The solution of enol ethers was added to the reaction mixture and the resulting mixture was stirred for an additional hour. Water (200 mL) was added and the product was extracted with CH_2Cl_2 (4 × 100 mL). The combined extracts were dried over $MgSO_4$, filtered through Celite, and concentrated. The residue was purified by column chromatography on SiO_2 eluted with CH_2Cl_2 to give 1.98 g (67%) of the telluropyranone **5b** as a brown solid: mp 130−131 °C; ^{1}H NMR ($CDCl_3$) δ 7.51−7.40 (m, 7 H), 7.31 (s, 2 H), 6.72 ("doublet" AA′BB′, 2 H, J = 9 Hz), 3.02 (s, 6 H); ^{13}C NMR ($CDCl_3$) δ 188.3, 151.9, 150.1, 148.0, 141.1, 132.7, 130.0, 129.4, 128.7, 128.1, 127.7, 126.8, 112.2, 40.1; IR (KBr) 3447, 2894, 1586; EIMS *m/z* 405 ($C_{19}H_{17}NO^{130}Te$). Anal. ($C_{19}H_{17}NOTe$) C, H, N.

2,4-Di[4-(dimethylamino)phenyl]-6-phenylthiopyrylium Chloride (2h). A mixture of 4-bromo-*N*,*N*-dimethylaniline (0.520 g, 2.60 mmol) and magnesium (0.032 g, 1.30 mg-at) was heated at reflux in THF (20 mL) for 3 h. A solution of thiopyranone **5b** (0.200 g, 0.651 mmol) in 10 mL of anhydrous THF was added dropwise to the reaction mixture and the resulting solution was heated at reflux for an additional hour. The reaction mixture was concentrated. The residue was dissolved in acetic acid (10 mL) and added dropwise to 10% HPF_6 (10 mL) at 0 °C. The product was collected by filtration and washed with water (4 × 10 mL) and ether (4 × 10 mL). The solid was redissolved in a small amount of acetone, added to H_2O (200 mL), and recollected by filtration. The crude product was dissolved in 10 mL of methanol and 0.3 g of Amberlite IRA-400 Cl ion-exchange resin was added. The resulting mixture was stirred 0.5 h and the exchange resin was removed via filtration. The process was repeated with two additional 0.3-g aliquots of the ion-exchange resin. The product was recrystallized from CH_3CN to give 0.203 g (90%) of **2h**: mp 238−240 °C; ^{1}H NMR (CD_2Cl_2) δ 8.38 (s, 1 H), 8.26 (s, 1 H), 8.01 (d, 2 H), 7.98−7.64 (m, 7 H), 6.91−6.84 (m, 4 H), 3.20 (s, 6 H), 3.17 (s, 6 H); λ_{max} (CH_2Cl_2) 627 nm [ϵ (8.7 ± 0.3) × 10^4 M^{-1} cm^{-1}]. Anal. as the hexafluorophosphate salt ($C_{27}H_{27}F_6N_2PS$) C, H, N.

2,4-Di[4-(dimethylamino)phenyl]-6-phenylselenopyrylium Chloride (3h). A mixture of 4-bromo-*N*,*N*-dimethylaniline (0.452 g, 2.26 mmol) and magnesium (0.054 g, 2.3 mg-at) was heated at reflux in THF (20 mL) for 3 h. A solution of selenopyranone **5b** (0.200 g, 0.564 mmol) in 10 mL of anhydrous THF was added dropwise to the reaction mixture and the resulting solution was treated as described for the preparation of **2h**. The product was recrystallized from CH_3CN to give 0.148 g (67%) of **3h**: mp >260 °C; ^{1}H NMR (CD_2Cl_2) δ 8.34 (s, 1 H), 8.26 (s, 1 H), 8.00 ("doublet" AA′BB′, 2 H, J = 9 Hz), 7.83−7.65 (m, 5 H), 7.62 (m, 2 H), 6.93−6.84 (m, 4 H), 3.19 (s, 6 H), 3.16 (s, 6 H); λ_{max} (CH_2Cl_2) 657 nm [ϵ (5.5 ± 0.2) × 10^4 M^{-1} cm^{-1}]. Anal. as the hexafluorophosphate salt ($C_{27}H_{27}F_6N_2PSe$) C, H, N.

2,4-Di[4-(dimethylamino)phenyl]-6-phenyltelluropyrylium Chloride (4h). A mixture of 4-bromo-*N*,*N*-dimethylaniline (0.396 g, 1.98 mmol) and magnesium (0.024 g, 0.99 mg-at) was heated at reflux in THF (20 mL) for 3 h. A solution of telluropyranone **5b** (0.200 g, 0.496 mmol) in 10 mL of anhydrous THF was added dropwise to the reaction mixture and the resulting solution was treated as described for **2h**. The product was recrystallized from CH_3CN to give 0.234 g (89%) of **4h**: mp 250−252 °C; ^{1}H NMR ($CDCl_3$) δ 8.32 (s, 2 H), 7.98 ("doublet" AA′BB′, 2 H, J = 9 Hz), 7.68−7.66 (m, 7 H), 6.92 ("doublet" AA′BB′, 2 H, J = 9 Hz), 6.85 ("doublet" AA′BB′, 2 H, J = 9 Hz), 3.16 (s, 6 H), 3.15 (s, 6 H); λ_{max} (CH_2Cl_2) 689 nm [ϵ (5.4 ± 0.02) × 10^4 M^{-1} cm^{-1}]. Anal. as the hexafluorophosphate salt ($C_{27}H_{27}F_6N_2PTe$) C, H, N.

2-[4-(Dimethylamino)phenyl]-4,6-diphenylthiopyrylium Chloride (2i). Phenylmagnesium bromide (1.09 mL of a 3.0 M solution in ether) was added dropwise to a solution of thiopyranone **5b** (0.200 g, 0.651 mmol) in 20 mL of anhydrous THF (10 mL) for 3 h. The resulting solution was heated at reflux for an additional hour. The reaction mixture was concentrated. The residue was dissolved in acetic acid (10 mL) and added dropwise to 10% HPF_6 (10 mL) at 0 °C. The product was collected by filtration and washed with water (4 × 10 mL) and ether (4 × 10 mL). The solid was redissolved in a small amount of acetone, added to H_2O (200 mL), and recollected by filtration. The crude product was dissolved in 10 mL of methanol and 0.3 g of Amberlite IRA-400 Cl ion-exchange resin was added. The resulting mixture was stirred for 0.5 h and the exchange resin was removed via filtration. The process was repeated with two additional 0.3-g aliquots of the ion-exchange resin. The product was recrystallized from CH_3CN to give 0.102 g (84%) of **2i**: mp 218−220 °C; ^{1}H NMR (CD_2Cl_2) δ 8.47 (s, 1 H), 8.26 (s, 1 H), 8.02−7.86 (m, 5 H), 7.69 (m, 7 H), 6.92 ("doublet" AA′BB′, 2 H, J = 9 Hz), 3.24 (s, 6 H); λ_{max} (CH_2Cl_2) 624 nm [ϵ (2.5 ± 0.1) × 10^4 M^{-1} cm^{-1}]. Anal. as the hexafluorophosphate salt ($C_{25}H_{22}F_6NPS$) C, H, N.

2-[4-(Dimethylamino)phenyl]-4,6-diphenylselenopyrylium Chloride (3i). Phenylmagnesium bromide (0.94 mL of a 3.0 M solution in ether) was added dropwise to a solution of selenopyranone **5b** (0.200 g, 0.564 mmol) in 10 mL of anhydrous THF. The resulting solution was treated as described for the preparation of **2i**. The product was recrystallized from CH_3CN to give 0.170 g (67%) of **3i**: mp 243−245 °C; ^{1}H NMR ($CDCl_3$) δ 8. 33 (s, 1 H), 8.19 (s, 1 H), 7.97−7.66 (m, 10 H), 6.93 ("doublet" AA′BB′, 2 H, J = 9 Hz) 3.24 (s, 6 H); λ_{max} (CH_2Cl_2) 654 nm [ϵ (3.4 ± 0.2) × 10^4 M^{-1} cm^{-1}]. Anal. as the hexafluorophosphate salt ($C_{25}H_{22}F_6NPSe$) C, H, N.

2-[4-(Dimethylamino)phenyl]-4,6-diphenyltelluropyrylium Chloride (4i). Phenylmagnesium bromide (0.83 mL of a 3.0 M solution in ether) was added to a solution of telluropyranone **5b** (0.200 g, 0.496 mmol) in 10 mL of anhydrous THF. The resulting solution was treated as described for the preparation of **2i**. The product was recrystallized from CH_3CN to give 0.220 g (73%) of **4i**: mp 243−244 °C; ^{1}H NMR [500 MHz] ($CDCl_3$) δ 8.22 (d, 2 H), 7.83 (dd, 4 H), 7.71−7.62 (m, 8 H), 6.91 (dd, 2 H) 3.20 (s, 6 H); λ_{max} (CH_2Cl_2) 693 nm [ϵ (3.5 ± 0.1) × 10^4 M^{-1} cm^{-1}]. Anal. as the hexafluorophosphate salt ($C_{25}H_{22}F_6NPTe$) C, H, N.

Electrochemical Procedures. A Princeton Applied Research Model 173 potentiostat/galvanostat and a model 175 universal programmer were used for the electrochemical measurements. The working electrode for cyclic voltammetry

was a platinum disk electrode (diameter, 1 mm) obtained from Princeton Applied Research. The auxiliary electrode was a platinum wire. The reference for cyclic voltammetry was the Fc/Fc^+ couple at +0.40 V at a scan rate of 0.1 V s^{-1}. All samples were run in J.T. Baker HPLC-grade dichloromethane that had been stored over 3A molecular sieves. Electrometric-grade tetrabutylammonium fluoroborate (Southwestern Analytical Chemicals, Inc.) was recrystallized from ethyl acetate−ether and then dried overnight at 80 °C before it was used as supporting electrolyte at 0.2 M. Argon was used for sample deaeration.

Quantum Yields for Singlet Oxygen Generation. The singlet oxygen acceptor 1,3-diphenylisobenzofuran (DPBF), HPLC-grade methanol, and certified rose bengal and methylene blue were used as received from Aldrich Chemical Co. Quantum yields for singlet oxygen generation in air-saturated methanol were determined by monitoring the dye-sensitized photooxidation of DPBF. DPBF is a convenient acceptor since it absorbs in a region of dye transparency and rapidly scavenges singlet oxygen to give colorless products. This reaction occurs with little or no physical quenching.[29] The indirect studies of quantum yields of singlet oxygen generation were carried out in a stopped-flow spectrophotometer (Applied Photophysics, Ltd.; ×18). The output beam of a 150-W Xenon arc lamp was passed through a monochromator (0.30-mm slits) to separate the 410-nm radiation and to direct it to the mixing chamber (approximate volume of 100 μL) via a flexible optical fiber to provide the excitation for DPBF. The excitation beam entered the chamber through a port that provides a 2-mm path length. The fluorescence of DPBF was monitored at a 90° angle to the excitation beam via a PMT (Hamamatsu) close-coupled to the chamber assembly. The fluorescence was passed through a 460-nm interference filter (Oriel) placed in front of the PMT window. The photolysis radiation was supplied by a 100-W QTH lamp (Oriel). The output of the lamp was passed through 590-nm long-pass and 660-nm interference filters (both from Oriel) and focused onto an input window of a flexible optical fiber. The photolysis light via the fiber reached the mixing chamber by entering through the 10-mm path length port. The solution of the sensitizer and the solution of DPBF were prepared separately to prevent sample degradation and were injected into the mixing chamber that was kept at (25.0 ± 0.1) °C via a circulating water bath. The decay of fluorescence of DPBF was then observed on a 200- or 2000-s time scale. The fluorescence decays were analyzed using the routines provided with the stopped-flow instrument software package by fitting them to a first-order exponential decay function with a floating endpoint. A total of three or more measurements were recorded for each sample and the results averaged to obtain a mean value for the decay rate.

On the basis of our earlier work,[6a] methylene blue has a singlet oxygen quantum yield [$\Phi(^1O_2)$] of 0.50 in methanol, which is close to the quantum yield of 0.52 determined for methylene blue in both ethanol and water.[30] The quantum yield for singlet oxygen generation by dyes **2**−**4** may be calculated by comparing the rates of the decay for the dye of interest and methylene blue (MB) at the same DPBF concentration and optical density at 660 nm:

$$\Phi(^1O_2)(\text{dye}) = [\Phi_{ox,DPBF}(\text{dye}) \times \Phi(^1O_2)(\text{MB})]/\Phi_{ox,DPBF}(\text{MB})$$

The rate of disappearance of DPBF can be expressed as follows:

$$-\text{d[DPBF]}/\text{d}t = \{I_a \times \Phi(^1O_2) \times k_r[\text{DPBF}]\}/\{k_r[\text{DPBF}] + k_d\}$$

where k_r is the rate of reaction of DPBF with singlet oxygen, k_d is the rate of decay of singlet oxygen, and I_a is the absorbed light.[31] At low concentrations of DPBF, where $k_r[\text{DPBF}] \ll k_d$ (DPBF] < 3 μM, $k_d = 1.0 \times 10^{-5}$ s^{-1},[32] first-order decay kinetics were observed.

The value of $\Phi(^1O_2)$ for **2** was confirmed with rose bengal where $\Phi(^1O_2) = 0.76$.[33] Dye **2** gave $\Phi(^1O_2)$ of 0.020 ± 0.004 in comparisons to rose bengal, which is identical to the value obtained with methylene blue.

Determination of Partition Coefficients. The octanol/water partition coefficients were all measured at pH 7.4 in phosphate-buffered saline using UV−visible spectrophotometry. The measurements were done using a "shake flask" direct measurement.[34] Three to five minutes of mixing was followed by 1 h of settling time. Equilibration and measurements were made at 23 °C using a Perkin-Elmer Lambda 12 spectrophotometer. HPLC grade 1-octanol was obtained from Sigma-Aldrich.

Cells and Culture Conditions. CX-1, PC-3, and MDA-435 cell lines were grown in a 50:50 mix of Dulbecco's modified Eagle's medium (DMEM) and RMPI 1640 medium (GIBCO Laboratories, Grand Island, NY) supplemented with 10% calf serum (Hyclone Laboratories, Inc., Logan, UT) and antibiotics at 37 °C under 5% CO_2, 95% air, and 100% humidity. PC-3 (human prostate carcinoma) and MDA-435 (human breast carcinoma) cells were obtained from Dr. L. Nadler (Dana-Farber Cancer Institute), and CX-1 (human colon carcinoma) was from Dr. M. Wolpert (National Cancer Institute).

Colo-26 cells were maintained in RPMI 1640 supplemented with 10% fetal calf serum and antibiotics. The cells were incubated at 37 °C, 5% CO_2 and were split every 2−3 days.

In Vitro Clonogenic Assay. Cells were seeded at 1500 cells/well for CX-1, PC-3, and MDA-435 cells in 96-well plates (Becton Dickinson Labware, Lincoln Park, NJ). The assay was performed in duplicate. Dyes **2**−**4** were first dissolved in dimethyl sulfoxide, to prepare 10 mg/mL stock solutions. The final drug solution was made by mixing 100 μL of this stock solution with 10 mL of 5% CS DME media solution. On the following day, cells were treated with test compounds at varied concentrations and cultured precisely for 24 h in the media. After rinsing, cells were incubated in drug-free medium for 2 weeks. Colonies were stained with 2% crystal violet in 70% ethanol and counted by an automated colony counter (Artek counter model 880, Dynatech Laboratories, Inc., Chantilly, VA).

In Vitro Phototoxicity Measurements. Colo-26 cells were plated at 2.5×10^4 cells/well of a 96-well tissue culture plate the evening before the assay. The day of the assay, the cells were washed twice with PBS, and 100 μL of HBSS, containing 1 μM **3a** or **3d**, was added to each well. The dye and cells were incubated for 2 h at 37 °C, followed by a wash with PBS and the addition of 100 μL of PBS. The plates were irradiated with filtered 590−800-nm light at 30 mW cm^{-2} with various light doses, and 100 μL of growth media was added. Cells were then incubated overnight at 37 °C, 5% CO_2. Cell survival was monitored using the MTT assay as described in Mosmann.[35]

Statistical Analyses. All statistical analyses were performed using the Student's *t*-test for pairwise comparisons. A P value < 0.05 was considered significant.

Animals. All animals were cared for under the guidelines of the Roswell Park Cancer Institute Committee on Animal Resources. Stock solutions of dyes **2**−**4** in 1% Tween 80/0.9% NaCl were prepared by dissolving the dye in Tween 80 and diluting to the appropriate volume with 0.9% NaCl. Animals were give 100-μL aliquots of the stock solutions via tail-vein injection.

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Supporting Information Available: Table 2 for IC_{50} values against several human carcinoma cell lines. This material is available free of charge via the Internet at http://pubs.acs.org.

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