Design, Synthesis and Photochemical Reactivation of Caged Prodrugs of 8-Hydroxyquinoline-Based Enzyme Inhibitors

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8-Hydroxyquinoline (HQ)-based compounds have recently been proposed as potential candidates for drugs for treating human immunodeficiency virus (HIV), cancer, neurodegenerative diseases (Alzheimer’s and Parkinson’s disease), and parasitic and bacterial infections. However, HQ itself and its derivatives might be toxic due to their intrinsic affinity for metal cations in living systems. One possible strategy for suppressing the toxicity and side effects of drugs with metal chelation properties, such as HQ, would be masking the critically important moieties with protecting groups that can be subsequently removed under specific conditions. In our previous work, we reported that HQ analogs are potent and selective inhibitors (Ki values=0.16–29μM) of aminopeptidase from Aeromonas proteolytica (AAP) (EC 3.4.11.10), a dinuclear Zn2+-peptidase. Based on this background information, HQ sulfonates were synthesized as prodrugs of HQ-based AAP-inhibitors that can be reactivated by photochemical cleavage of the S–O bond in the sulfonate groups. The findings indicate that HQ sulfonates containing methanesulfonyl and 2-aminoethanesulfonyl groups are essentially stable under physiological conditions and undergo photolysis to regenerate the corresponding HQ compounds that function as AAP inhibitors. This methodology could be applied to the design of similar types of Zn2+-hydrolase inhibitors and prodrugs.

Key words 8-hydroxyquinoline; prodrug; inhibitor; photolysis; Zn2+-peptidase

8-Hydroxyquinoline (HQ)-based compounds have recently been proposed as potent protease-inhibitors1–3 and potential candidates for drugs for use in the treatment of AIDS, cancer, neurodegenerative diseases (Alzheimer’s and Parkinson’s disease), parasitic or bacterial infections.4–13 5-Chloro-8-hydroxy-7-iodoquinoline (clioquinol, Chart 1) has been clinically used as an antifungal and antiprotozoal drug prior to the 1970’s.14 The use of clioquinol was, however, banned after the 1970’s, because it was suspected that it might cause serious side effects called subacute myelo-optico neuropathy, SMON. Although the mechanism responsible for the development of SMON is not fully understood, one of its causes has been attributed to its ability to form complexes with metal ions such as Fe2+ in the human body.15 Recently, however, this situation seems to be changing somewhat, as mentioned above. As a typical example,16 5,7-dichloro-8-hydroxyquinoline (chloroxine, Chart 1), a topical antimicrobial agent, has been reported to be an inhibitor of biofilm formation of Candida albicans17 and human platelet type 12-lipoxygenase.18

It was reported that so-called “caged” drugs, particularly those containing photocleavable protecting groups, play important roles in the control of bioactivities.19–26 As general (not limited to metal chelators) examples of “caged” biologically active compounds, Hoffman’s group reported the design and synthesis of “caged ATP,” which is an ATP molecule that is protected by a photocleavable (2-nitro) phenylethyl group, to investigate the function of the Na+:K pump in human red blood cells.19 Hess’ group also reported on the use of N-nitrobenzyl serotonin, a “caged serotonin,” in a kinetic study of the serotonin 5-HT1 receptor.23 The advantage of photolabile groups has been applied to caged-metal chelators27–30 such as ethylene glycol bis(2-aminoethy ether)-N,N’N’’N’’’-tetraacetic acid (EGTA) and ethylenediaminetetraacetic acid (EDTA) analogues that are protected by o-nitrophenyl groups, and can be reactivated to form metal complexes, as reported by Kaplan.28 Therefore, one of the strategies for suppressing the toxicity and side effects of metal chelator-based drugs such as HQ would be masking the critically important moieties with protecting groups that can be removed under specific conditions.

In our previous work, we reported that HQ 1 and its analogs (2–8) are potent and selective inhibitors (Ki values=0.16–29μM) of an aminopeptidase from Aeromonas proteolytica (AAP) (EC 3.4.11.10), a dinuclear Zn2+-peptidase (Chart 2). They have negligible inhibitory activities against leucine aminopeptidase (LAP), alkaline phosphatase, carboxypeptidase A, and carbonic anhydrase (CA)31 (Chart 2). The X-ray crystal structure of the AAP–1 complex disclosed that these HQ inhibitors bind to the dinuclear Zn2+ active site via the coordination of 8-O− to two Zn2+ ions and that of a nitrogen atom to one Zn2+. On the other hand, 2-methyl-8-hydroxyquinoline 9 has negligible inhibitory activity against AAP, possibly due to the steric hindrance of the methyl group at the 2 position.

We also reported that the HQ sulfonates undergo S–O bond cleavage reactions by photirradiation in aqueous solutions to afford the corresponding 8-quinolinols and sulfonates with negligible byproduct production.32–36

For the purpose of reducing the side effects of HQ-based agents not only for AAP inhibitors but also for other HQ-

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Chart 1. Structures of Clioquinol and Chloroxine

\[\begin{align*}
\text{Cl} & \quad \text{OH} \\
\text{X} & = \text{I: clioquinol} \\
\text{X} & = \text{Cl: chloroxine}
\end{align*}\]
based drugs and homologues, we were prompted to design and synthesize prodrugs of HQ that can be activated by photoirradiation as an external trigger. Although carboxyl ester was frequently chosen as protecting group for caged compounds, the carboxyl esters of HQ were unstable under physiological conditions. Recently, only a few papers have appeared on sulfonates as prodrugs that are more stable than carboxyl esters and can be reactivated under the condition in the presence of H$_2$O$_2$ or hypoxia conditions.

In this paper, we report on the design and synthesis of HQ methanesulfonates (Chart 3) as prodrugs of HQ-type drugs. 1, 3, 6 and 7 were chosen as active forms of these prodrugs, and 9 was chosen as the control compound. The methanesulfonyl group, which has the smallest steric hindrance, was used as a protecting group. 2-Aminoethanesulfonate group was also introduced, because 2-aminoethanesulfonate derivatives should be more hydrophilic than methanesulfonates and the byproduct of the photoreaction is 2-aminoethanesulfonic acid (taurine) that is considered to be nontoxic. The photochemical unmasking reactions of these prodrugs were evaluated using AAP and these results are also discussed.

Results and Discussion

**Synthesis of Methanesulfonates and 2-Aminoethanesulfonate Derivatives of HQ as Prodrugs of AAP Inhibitors**

The preparation of 10 was carried out according to literature methods and 5-substituted HQ methanesulfonates 11–13 were prepared from the corresponding 8-quinolinols and methanesulfonyl chlorides (see Experimental). For 14–16, the tetrabutylammonium salt 18 prepared from 17 according to a literature procedure was converted to the corresponding sulfonyl chloride, and then condensed with the HQ to afford 19, 20 and 21 (Chart 4). Treatment of 19–21 with HCl in dioxane gave 14–16, respectively, as HCl salts.

**Stability of Prodrugs in Aqueous Condition**

We checked the stability of 10–16 in aqueous solution at pH 8.0 (10 mM 3-[4-(2-hydroxyethyl)-1-piperazinyl]propanesulfonic acid (EPPS) with I=0.1 (NaNO$_3$) containing 2% CH$_3$CN and 37°C, enzymatically. These compounds were incubated at pH 8.0 for a given period of time (Xh) in the absence of AAP, and the hydrolysis of leucine p-nitroanilide (LeuNA) by AAP at the same pH was followed by monitoring the absorption of p-nitrophenylaniline (405 nm) that is produced by the hydrolysis of LeuNA (Fig. 1). These data indicate that 13, 14 and 15 are slowly hydrolyzed, releasing the corresponding...
8-quinolinols, 1, 3 and 7, that have AAP inhibitory activity. Because the \( K_i \) values of 1, 3 and 6, the original compounds of 10–12, are 0.19–0.68 \( \mu M \), respectively, the results shown in Fig. 1 indicate that less than 1% of compounds 10–12 are hydrolyzed. Although it is likely that 9 is produced from 16, negligible AAP inhibition was observed, possibly due to the high \( K_i \) value (\( >100 \mu M \)) of 9 (see Chart 2). These results indicate that the HQ methanesulfonate derivatives 10–12 are very stable under physiological conditions.

Reactivation of HQ Sulfonate Derivatives by Photoirradiation We examined the reactivation of HQ sulfonates, 10–16 by the photochemical cleavage of their S–O bonds. First, the generation of the corresponding HQ (1 mM) by photoirradiation at >330 nm in a 50/50 DMSO-\( d_6/D_2O \) solution (10 mM EPPS (pD 8.0) at 25°C) was monitored by \(^1\)H-NMR. As shown in Fig. 2, 3 is regenerated from 11 with negligible side reactions. The photochemical reactivation of 10, 11, 13 and 15 was also followed by UV-Vis spectra (Fig. 3). As shown in Fig. 3, 13 was activated very slowly by photoirradiation, and the photoreactivities of 10, 11 and 15 are almost identical (the quantum yields for the photolysis (\( \Phi \)) of 10, 11, 13 and 15 were 7.3\( \times 10^{-5} \), 9.9\( \times 10^{-5} \), 5.8\( \times 10^{-6} \) and 8.3\( \times 10^{-5} \), respectively).

We next examined the inhibition of AAP by HQ that was regenerated by photoirradiation, the experimental protocols of which are summarized in Chart 5 (Methods A, B, C). In Method A, reaction mixtures of 10–16 (5 \( \mu M \)) and AAP were photoirradiated at 313 nm at pH 8.0 (10 mM EPPS with \( I=0.1 \) (NaNO\(_3\)) containing 2\% CH\(_3CN\)) and 25°C for \( Y \) min (\( Y=1, 3, 5, 10, 15 \)). LeuNA was then added and its hydrolysis was followed by monitoring the change in absorption at 405 nm due...
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to the release of p-nitroaniline, as described above. In Methods B and C, the hydrolysis of LeuNA by AAP was measured after irradiation of the HQ sulfonates alone and AAP alone to evaluate the effect of photoirradiation on AAP.

In Figs. 4a–f, it is indicated that the AAP activity in Method C (open circle in Fig. 4) negligibly changes after photoirradiation. Besides, the plots in Method A and Method B are almost superimposable in Figs. 4a–f. These data strongly suggest that AAP is essentially not damaged by photoirradiation. After the photoirradiation of 10, 11, 14, and 15 for 15 min, the hydrolytic activities of AAP were inhibited by more than 50% (Figs. 4a, b, d, e). In particular, the AAP-activity in the presence of 11 was decreased to ca. 30% by photoirradiation for only 5 min (Fig. 4b). Note that 13 and 16 are exceptions (Figs. 4c, f), because their photochemical products, 7 and 9, have larger Ki values than the other HQ’s. Although AAP-inhibition was observed in the presence of 12 after photoirradiation, the results of AAP inhibition were not reproducible (data not shown), possibly due to slow binding property of 6 to AAP, as we previously reported.31 These results indicate that the inhibition against AAP after photolysis can be directly attributed to the corresponding HQ derivatives generated by the reactivation of HQ sulfonates. It should also be noted that the inhibitory activity of the photochemical products from HQ 2-aminoethanesulfonates 14 and 15 against AAP is less potent than that of the corresponding HQ methanesulfonates 10 and 11, which could be due to some interactions (electrostatic, hydrophobic or other interactions) between 14 or 15 and the enzyme. The stronger inhibition of 11 and 15 in comparison with the other prodrugs (Fig. 3) is attributable to the fact they have higher Φ values than other prodrugs and the smallest Ki values of the parent compound 3 (Chart 2).

Conclusion

We report herein on the design and synthesis of HQ sulfonates 10–16 for use as prodrugs of HQ-based AAP-inhibitors and their reactivation by photolysis. The findings show that HQ methanesulfonates 10 and 11 are stable under physiological conditions. The photoirradiation of HQ sulfonates 10, 11, 14 and 15 promotes the cleavage of the S–O bond of these prodrugs, resulting in the regeneration of the corresponding HQ derivatives that function as inhibitors of AAP. The most efficient inhibition of AAP was observed by photoreactivation of 11, possibly due to the fact that it undergoes photocleavage most rapidly and has the smallest Ki values of 3, which is the original compound of 11. Although the reactivation efficiency of the HQ sulfonates in this paper is somewhat low, this “caging” strategy would be useful for the HQ type and related inhibitors of not only AAP but also other metal enzymes and proteins. The substitutions of the HQ derivatives can con-

Fig. 4. Results for the Photochemical Reactivation of 10 (a), 11 (b), 13 (c), 14 (d), 15 (e) and 16 (f) (for Methods A (Open Triangles), B (Open Squares), and C (Open Circles), See Text and Chart 5)

Irradiation at 313 nm for Y min at pH 8.0 (10 mM EPPS with I=0.1 (NaNO3) containing 2% CH3CN) and 25°C. Initial concentration of the prodrugs is 5 μM.
tribute to the acceleration of photochemical S–O bond cleavage reaction, the red-shift of the irradiation wavelength and the improvement of inhibition of Zn²⁺ enzymes such as β-lactamases. These results indicate that HQ sulfonates represent potential candidates for produgs of the inhibitors against AAP and that these methods can be applied to the design of similar types of Zn²⁺ hydrolase inhibitors and produgs that could be used in the treatment of HIV, cancer, neurodegenerative diseases, and parasitic and bacterial infections.

Experimental

General Information All reagents and solvents purchased were of the highest commercial quality and were used without further purification. The aminopeptidase from AAP was purchased from Sigma-Aldrich. All aqueous solutions were prepared using deionized and distilled water. The Good’s buffer reagents (Dojindo) were commercially obtained: EPPS. Melting points were determined on a Yanaco Melting Point Apparatus and a Büchi 510 Melting Point Apparatus and are uncorrected. UV-Vis spectrographs were recorded on a JASCO UVD-2401PC spectrophotometer. 1H- and 13C-NMR spectra were recorded on a JEOL JNM-ECS400 spectrometer. 1H-NMR (CDCl₃) δ: 3.52 (3H, s), 7.66 (1H, dd, J = 4.1, 9.0 Hz), 7.98 (1H, d, J = 8.1 Hz), 8.57 (1H, d, J = 8.7 Hz), 9.08 (1H, d, J = 3.6 Hz). 13C-NMR (CDCl₃) δ: 39.6, 121.7, 122.0, 123.3, 125.4 (q, J = 5.6 Hz), 125.7, 126.2, 133.0 (q, J = 2.5 Hz), 141.7, 148.5, 151.4. HR-FAB-MS m/z: 292.0255 (Calcd for C₃₃H₂₃F₃NO₄S [M+H]+: 292.0253).

Synthesis of HQ 2-Aminomethanesulfonates 14, 15 and 16 HQ 2-Aminomethanesulfonate Hydrochloride Salt (14) A solution of N-Boc-2-aminomethanesulfonate nBu₂N⁺ salt (18) (1877 mg, 4.02 mmol) in CH₂Cl₂ (6.0 mL) was treated with N,N-dimethylformamide (DMF) (0.031 mL, 0.40 mmol) and then with triphosgene (477 mg, 1.61 mmol) at room temperature. After stirring at room temperature for 1h, the reaction mixture was cooled to 0°C, to which a solution of Et₃N (1.20 mL, 8.06 mmol), 4-dimethylaminopyridine (DMAP) (cat.) and HCl in dioxane (0.5 mL) was added dropwise (10 min). The resulting solution was stirred overnight at room temperature, the phosgene was then removed by bubbling with Ar, and the solution was diluted with CH₂Cl₂ (6 mL). The reaction mixture was washed with a saturated aqueous NH₄Cl solution (3×10 mL) and brine (10 mL). The organic layer was dried anhydrous Na₂SO₄, filtered, and evaporated. The crude product was purified by column chromatography on silica gel with hexanes:AcOEt from 10:1 to 5:1, followed by reprecipitation from CH₂Cl₂/hexanes to afford the product as colorless solid, with 7% yield as colorless solid, which was used without further purification in the next step.

IR (neat) cm⁻¹: 3223, 3052, 2970, 1698, 1579, 1499, 1368, 1276, 1165, 1056, 1016, 983, 892, 796, 722, 771, 726. 1H-NMR (CDCl₃) δ: 1.46 (9H, s), 3.69 (2H, t, J = 5.8 Hz), 7.70–7.76 (2H, m), 8.10 (2H, d, J = 8.1 Hz). 13C-NMR (CDCl₃) δ: 34.1, 48.5, 122.8, 123.2, 126.7, 129.6, 137.2, 140.0, 143.8, 151.5. HR-FAB-MS m/z: 353.1170 (Calcd for C₁₆H₁₈N₂O₅S [M+H]+: 353.1171).

4× HCl in dioxane (0.5 mL) was added to a solution of 19 (33 mg, 0.093 mmol) in 1,4-dioxane (0.5 mL). After stirring the reaction mixture for 10 min, the solvent was removed on a rotary evaporator to give the crude product, which was purified by reprecipitation from MeOH/Et₂O to give 14 (23 mg, 0.071 mmol, 76% yield) as colorless solid.

IR (neat) cm⁻¹: 3289, 2830, 2739, 2662, 2085, 1602, 1549, 1369, 1351, 1297, 1288, 1180, 1165, 1123, 1077, 1035, 995, 891, 872, 824, 759, 744. 1H-NMR (CDCl₃) δ: 3.80 (2H, t, J =5.8 Hz), 4.17 (2H, t, J = 5.8 Hz), 7.70–7.76 (2H, m), 8.10 (1H, d, J = 8.1 Hz), 8.55 (1H, d, J = 8.1 Hz), 8.97 (1H, d, J = 4.6 Hz). 13C-NMR (CDCl₃) δ: 34.1, 48.5, 122.8, 123.2, 126.7, 129.6, 137.2, 140.0, 143.8, 151.5. HR-FAB-MS m/z: 253.0647 (Calcd for C₁₆H₁₈N₂O₅S [M+H]+: 253.0647).

Synthesis of HQ 2-Aminomethanesulfonates 11, 12 and 13 (29) Methanesulfonyl chloride (1.1 eq against HQ) and 4-dimethylaminopyridine (cat.) were added to a solution of the corresponding HQ 3, 6 or 7 (1 eq) and Et₃N (1.2 eq) in CH₂Cl₂, and the resulting solution was stirred at room temperature until the 8-quinolinols disappeared, as confirmed by TLC. After concentrating the reaction mixture under reduced pressure, the remaining residue was dissolved in AcOEt and the organic solution was washed with water and then brine. The organic layer was then dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The resulting residue was purified by column chromatography on silica gel hexanes:AcOEt (from 10:1 to 5:1) and recrystallization from hexanes:AcOEt.

11: mp: 97–98°C. IR (neat) cm⁻¹: 3018, 1591, 1495, 1463, 1346, 1174, 1143, 1051, 1032, 980, 810, 784. 1H-NMR (CDCl₃) δ: 3.47 (3H, s), 7.60–7.67 (3H, m), 8.63 (1H, dd, J = 1.7, 8.9 Hz), 9.03 (1H, dd, J = 1.7, 8.9 Hz). 13C-NMR (CDCl₃) δ: 36.6, 120.2, 120.9, 123.7, 124.9, 127.6, 130.8, 139.2, 141.8, 148.8. High resolution fast atom bombardment mass spectrometry (HR-FAB-MS) m/z: 257.9866 (Calcd for C₁₅H₁₄Cl₁NO₂S [M+H]+: 257.9992). Anal. Caled for C₁₅H₁₄Cl₁NO₂S: C, 46.61; H, 3.13; N, 5.44. Found: C, 46.69; H, 2.85; N, 5.46.

12: mp: 100–102°C. IR (neat) cm⁻¹: 3019, 1579, 1506, 1362, 1321, 1157, 1119, 1060, 1038, 970, 822, 792, 764, 732. 1H-NMR (CDCl₃) δ: 3.52 (3H, s), 7.66 (1H, dd, J = 4.1, 9.0 Hz), 7.98 (1H, d, J = 8.1 Hz), 8.57 (1H, d, J = 8.7 Hz), 9.08 (1H, d, J = 3.6 Hz). 13C-NMR (CDCl₃) δ: 39.6, 121.7, 122.0, 123.3, 125.4 (q, J = 5.6 Hz), 125.7, 126.2, 133.0 (q, J = 2.5 Hz), 141.7, 148.5, 151.4. HR-FAB-MS m/z: 292.0255 (Calcd for C₃₃H₂₃F₃NO₄S [M+H]+: 292.0253).
3 (1598 mg, 8.9 mmol) in a manner similar to that described for 19.

IR (neat) cm⁻¹: 3231, 2976, 1704, 1553, 1484, 1465, 1338, 1275, 1249, 1162, 1057, 948, 867, 827, 784, 705. ¹H-NMR (CDCl₃) δ: 1.46 (9H, s), 3.69–3.72 (2H, m), 3.88–3.93 (2H, m), 7.63–7.68 (2H, m), 7.74 (1H, d, J = 8.4 Hz), 8.65 (1H, dd, J = 1.5, 8.7 Hz), 9.03 (1H, d, J = 4.5 Hz). HR-FAB-MS m/z: 387.0825 (Calcd for C₁₂H₁₄N₂O₃S [M⁺]+: 387.0781).

5-Chloro-8-hydroxyquinoline 2-aminooethanesulfonate hydrochloride salt (15) was prepared as yellow crystal (131 mg, 36% yield) from 20 (38.6 mg, 0.10 mmol) in a manner similar to that described for 14.

mp: >200°C. IR (neat) cm⁻¹: 3487, 3429, 2887, 2043, 1634, 1588, 1503, 1468, 1361, 1300, 1223, 1180, 1038, 952, 902, 853, 789. ¹H-NMR (D₂O) δ: 3.79 (2H, t, J = 5.7 Hz), 4.18 (2H, t, J = 5.7 Hz), 7.77–7.84 (3H, m), 8.49, 7.78. ¹H-NMR (CDCl₃) δ: 3.40, 48.5, 123.4, 124.0, 126.7, 126.8, 129.3, 133.4, 140.9, 143.4, 152.4. HR-FAB-MS m/z: 287.0340 (Calcd for C₁₀H₁₀ClN₂O₃S [M⁺]+: 287.0256). Anal. Calcd for C₁₀H₁₀ClN₂O₃S·2HCl: C, 35.35; H, 3.75; N, 7.54. Found: C, 35.36; H, 3.75; N, 7.41.

2-Methyl-8-hydroxyquinoline 2-aminooethanesulfonate Hydrochloride Salt (16) 2-Methyl-8-hydroxyquinoline N-Boc-2-aminooethanesulfonate (21) was prepared as colorless needles (30.1 mg, 77% yield) from 18 (374.5 mg, 8.0 mmol) and 9 (1528 mg, 9.6 mmol) in a manner similar to that described for 19.

IR (neat) cm⁻¹: 3216, 2974, 1504, 1558, 1249, 1280, 1106, 1058, 983, 908, 849, 778. ¹H-NMR (CDCl₃) δ: 1.42 (9H, s), 2.79 (3H, s), 3.68–3.71 (2H, m), 3.90–3.95 (2H, m), 7.38 (1H, d, J = 8.7 Hz), 8.88 (1H, d, J = 8.4 Hz). ¹H-NMR (D₂O) δ: 1.42 (9H, s), 2.79 (3H, s), 3.68–3.71 (2H, m), 3.90–3.95 (2H, m), 7.38 (1H, d, J = 8.4 Hz). HR-FAB-MS m/z: 367.1328 (Calcd for C₁₂H₁₄N₂O₃S [M⁺]+: 367.1328).

2-Methyl-8-hydroxyquinoline 2-aminooethanesulfonate hydrochloride salt (16) was prepared as colorless needles (30.1 mg, 77% yield) from 21 (42.0 mg, 0.11 mmol) in a manner similar to that described for 14.

mp: >200°C. IR (neat, cm⁻¹): 3371, 2886, 2699, 1648, 1600, 1544, 1490, 1418, 1342, 1299, 1221, 1180, 1163, 1069, 1044, 910, 845, 798. ¹H-NMR (D₂O) δ: 3.82 (2H, t, J = 6.3 Hz), 4.29 (2H, t, J = 6.3 Hz), 7.85–7.91 (2H, m), 8.13 (1H, d, J = 7.2 Hz), 8.21 (1H, d, J = 8.1 Hz), 8.88 (1H, d, J = 8.7 Hz). ¹H-NMR (CDCl₃) δ: 22.4, 34.6, 48.5, 123.4, 124.0, 129.1, 129.2, 144.0, 144.1, 161.5, 161.6. HR-FAB-MS m/z: 367.0807 (Calcd for C₁₂H₁₀N₂O₃S [M⁺]+: 367.0803). Anal. Calcd for C₁₂H₁₀N₂O₃S·2HCl: C, 42.49; H, 4.75; N, 8.26. Found: C, 42.70; H, 4.69; N, 8.21.

Photoreactions Followed by NMR and UV-Vis Experiments In NMR experiments, a 5 m M solution of each HQ sulfonate, 25 µL of substrate (10 mM in CH₃CN) was added in the reaction mixture after photoirradiation for given periods (Y/min) and the change in absorption at 405 nm was recorded by means of a UV-Vis spectrometer to determine the initial velocity of the hydrolysis. In the case of prodrugs alone, 0.02 units of AAP and 25 µL of substrate (10 mM in CH₃CN) were added and the initial rate of hydrolysis was obtained in the same way.

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References and Notes

44) As reported in our previous publication (ref. 26), the hydrolysis of LeuNA follows Michaelis–Menten kinetics.
45) It should be noted that the photoreaction of 11 in 1H-NMR analysis required longer reaction time than that in UV-Vis analysis, because the concentration of 11 in 1H-NMR sample (5 mM) was much higher than that in UV-Vis sample (50 µM).
46) Quite recently, we have found HQ derivatives that have absorption maxima at ca. 380 nm. The details will be reported elsewhere.