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Functionalized quinolizinium-based fluorescent reagents for modification of cysteine-containing peptides and proteins†

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A series of quinolizinium-based fluorescent reagents were prepared by visible light-mediated gold-catalyzed *cis*-difunctionalization between quinolinium diazonium salts and electron-deficient alkyne-linked phenylethynyl trimethylsilanes. The electron-deficient alkynyl group of the quinolizinium-based fluorescent reagents underwent nucleophilic addition reaction with the sulphydryl group on cysteine-containing peptides and proteins. The quinolizinium-based fluorescent reagents were found to function as highly selective reagents for the modification of cysteine-containing peptides and proteins with good to excellent conversions (up to 99%). Moreover, the modified BCArg mutants bearing cationic quinolizinium compounds **1b**, **1d**, **1e** and **1h** exhibit comparable activity in enzymatic and cytotoxicity assays to the unmodified one.

Introduction

Functionalized organic fluorescent materials have been developed as powerful tools due to their wide applications in chemistry, biology and materials science.¹ Among the organic fluorescent scaffolds, fluorescein, rhodamine, coumarin, BODIPY, cyanine and, recently, Seoul-Fluor, have been intensively studied (Scheme 1a).² However, these fluorescent materials generally have poor water-solubility, leading to limitation of versatility, sensitivity and quantitative capabilities.³ Some of the cationic heterocycles such as isoquinolinium, cinnolinium, quinolizinium, indolizinium, benzimidazolium, azinium and acridinium have been developed as fluorophores to improve water-solubility for biological research,^{1c,4} but only a few examples have been reported on the unique properties of cationic fluorophores in photocatalysis^{1c,f,g} and cellular imaging.^{3b,c} Besides, these cationic fluorescent heterocycles were first studied as bioactive drug candidates and extensively employed as DNA intercalators.^{4b} However, applications in chemoselective modification of peptides and proteins using these cationic heterocycles remain sparse.

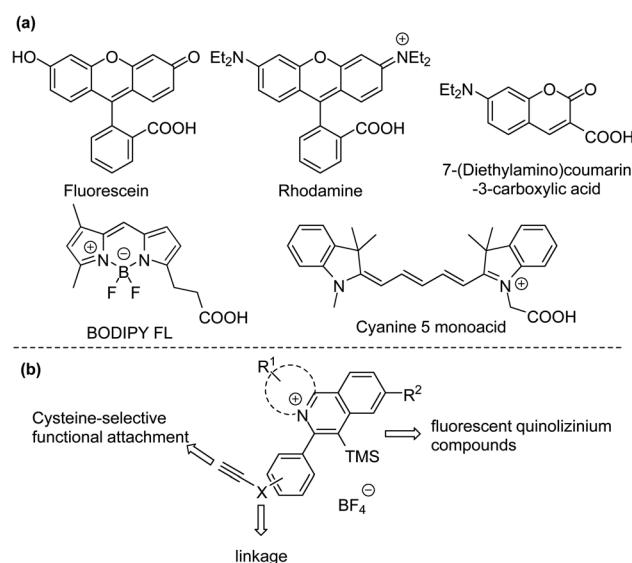
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Chemoselective chemical modification of peptides and proteins is of importance in chemical biology,⁵ which is useful to construct well-defined bioconjugates for biological studies and development of protein-based therapeutics and diagnostics.⁶ In the past decades, lysine and cysteine are targets-of-interest for chemical modification due to their high nucleophilicity.^{7,8} However, the high prevalence of lysine on protein



Scheme 1 (a) Selected examples of organic fluorescent dyes commonly used in bioconjugation reactions. (b) Our design of electron-deficient alkyne-containing quinolizinium compounds for cysteine modification.



surfaces leads to the formation of protein conjugates with a poor level of regioselectivity.^{5a} On the contrary, selective labeling of cysteine can be achieved by taking advantage of intrinsically low abundance (1.7%) and by incorporation using site-directed mutagenesis, rendering cysteine as an ideal target on protein bioconjugations. Conventional cysteine modifications heavily relied on *S*-alkylation with α -halocarbonyls and Michael addition to maleimides. Yet, owing to cross-reactivity, synthetic difficulties of α -halocarbonyls and hydrolytic instability of the maleimide-based conjugates, Davis *et al.*,⁹ Pentelute *et al.*,¹⁰ our group¹¹ and others¹² have reported cysteine selective modifications in metal-free and/or metal-mediated manners. Most recent advances demonstrated the use of hypervalent iodine reagents for cysteine modification.¹³ Despite these new achievements, the development of easily accessible and water-soluble reagents for cysteine selective modification under mild reaction conditions is still an ongoing interest.

Most recently, our group has developed a new series of quinolizinium compounds as versatile cationic fluorescent heterocycles.¹⁴ These quinoliziniums exhibit tunable photophysical properties in the visible light region and large Stokes shifts, enriching the applications of visible-light-induced photocatalysis,^{14a,b} cellular imaging^{14a,c} and molecular probes.^{14c} As such, the design of functionalized quinolizinium compounds by incorporation of the electron-deficient alkynyl group delivers easily accessible reagents for cysteine modification of peptides and proteins (Scheme 1). Along with our ongoing interest in the development of bioconjugation reactions,^{11,15} herein, we report a series of electron-deficient alkyne-linked quinolizinium compounds prepared from the visible light-mediated gold-catalyzed alkyne *cis*-difunctionalization. Photophysical properties of the newly developed quinolizinium compounds were also examined. We demonstrated that the fluorescent quinolizinium compounds could be used as efficient selective modification reagents for cysteine-containing peptides. The quinoliziniums also worked well for protein modification, including labelling a therapeutic *Bacillus caldovelox* arginase mutant (BCArg mutant). Enzymatic and anti-cancer activities of the modified BCArg mutants have also been studied.

Results and discussion

Synthesis, characterization and photophysical properties of quinolizinium-based fluorescent reagents

We first prepared a series of quinolizinium-based fluorescent reagents **1a–1h** using visible light-mediated gold-catalyzed alkyne *cis*-difunctionalization developed by our group (Fig. 1).^{14a} Using quinolizinium **1a** as an example, it can be prepared by treatment of quinoline-substituted aryl diazonium **3a** (0.12 mmol, 1.2 equiv.) and electron-deficient alkyne-containing phenylethynyl trimethylsilane **2a** (0.10 mmol, 1 equiv.) with Ph_3PAuCl (10 mol%) under irradiation of blue LEDs for 16 h. Electron-deficient alkyne-linked quinolizinium **1b–1h** were also prepared using the same method. Both alkynoic ketones **1a–1c** and alkynoic amides **1d–1h** were isolated in up to 49% yield after flash column purification. Our results revealed that electron-deficient alkynes have a high tolerance to the visible light-mediated gold catalysis reaction without losing the reactivity towards cysteine.

The photophysical properties of electron-deficient alkyne-linked quinoliziniums **1a–1h** were then measured by UV/Vis and fluorescence spectroscopies in dichloromethane (Table S3, ESI†). The results showed that the maximum absorption of the **1a–1h** was in the visible light region (λ_{abs} 405–445 nm) and the maximum emission was between 478–555 nm, with quantum yield (Φ_F) of up to 0.22. The molar absorptivity of these compounds was up to $15\,700\, \text{M}^{-1}\, \text{cm}^{-1}$. Moreover, alkynoic amides **1d–1h** showed slightly larger Stokes shift than alkynoic ketones **1a–1c**. In general, most of these findings were in line with our reported silyl-substituted quinoliziniums,^{14a} which demonstrated that this class of quinolizinium compounds could be amenable for the design of chemoselective reagents by incorporation of electron-deficient alkynes (alkynoic ketones and alkynoic amides) as one of the reactive functionalities towards peptide and protein labelling.

Quinolizinium compounds as cysteine selective reagents

Cysteine-containing peptide STSSCNLSK and quinolizinium reagent **1a** were set up as model substrates for condition screening with reference to our previous works.^{11a} In brief, by treatment of peptide STSSCNLSK (0.1 mM) with **1a** (1 equiv.) in 50 mM PBS (pH 7.4)/DMSO (90 : 10) at 25 °C for 6 h, **1a**-modified STSSCNLSK and dimerized STSSCNLSK were afforded in 89% and 6% conversions, respectively (entry 1, Table 1). The total ion chromatogram of the crude mixture of **1a**-modified STSSCNLSK by LC-MS analysis showed the modification was efficient (Fig. S2, ESI†). Selective attachment of **1a** to the cysteine sulphhydryl group of STSSCNLSK *via* nucleophilic addition was confirmed by LC-MS/MS (Fig. S10, ESI†). Neither the N-terminal α -amino group nor the side chains of serine, threonine and lysine were modified. Quinolizinium reagents **1b–1h** also worked well in the reaction with STSSCNLSK that gave 82–97% conversions (entries 2–8 and Fig. S11–S24, ESI†).

We then sought to demonstrate the chemoselectivity of the modification. Treatment of cysteine-containing peptides AYEMWCFQ, AYEMWCFQK, CSKFR, KSTFC and ASCGTN

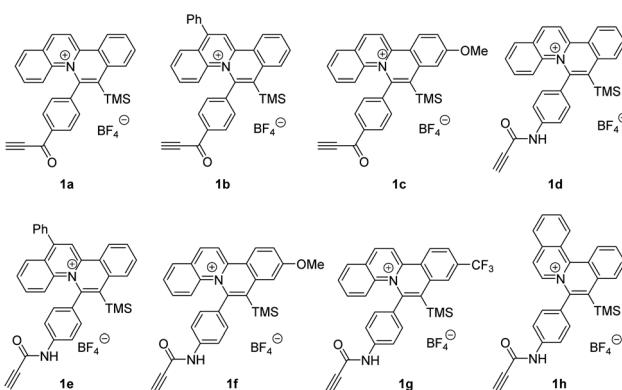
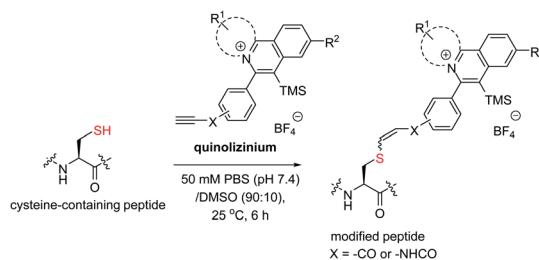


Fig. 1 Chemical structures of quinoliziniums **1a–1h**.



Table 1 Bioconjugation reaction of quinolizinium compounds with cysteine-containing peptides^a

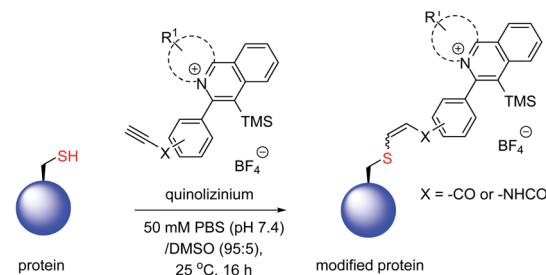
Entry	Peptide	Quinolizinium	Conversion ^b (%)
1	STSSSCNLSK	1a	89
2	STSSSCNLSK	1b	95
3	STSSSCNLSK	1c	82
4	STSSSCNLSK	1d	97
5	STSSSCNLSK	1e	94
6	STSSSCNLSK	1f	94
7	STSSSCNLSK	1g	85
8	STSSSCNLSK	1h	95
9	AYEMWCFHQR	1a	75
10	AYEMWCFHQR	1b	99
11	AYEMWCFHQR	1c	47
12	AYEMWCFHQR	1d	25
13	AYEMWCFHQR	1e	99
14	AYEMWCFHQR	1f	96
15	AYEMWCFHQR	1g	44
16	AYEMWCFHQR	1h	96
17	AYEMWCFHQK	1a	68
18	AYEMWCFHQK	1d	47
19	AYEMWCFHQK	1h	86
20	CSKFR	1a	73
21	CSKFR	1d	78
22	CSKFR	1h	63
23	KSTFC	1a	23
24	KSTFC	1d	39
25	KSTFC	1h	36
26	ASCGTN	1a	94
27	ASCGTN	1d	99
28	ASCGTN	1h	99

^a Treatment of cysteine-containing peptide (0.1 mM) with quinolizinium (1 equiv.) in 50 mM PBS (pH 7.4)/DMSO (90:10) for 6 h. ^b Determined by LC-MS analysis.

with quinolizinium compounds gave the corresponding modified peptides in up to 99% conversion (entries 9–28). MS/MS analysis revealed that only the cysteine residue was modified while other residues remained intact (Fig. S25–S64, ESI†).

Protein modification using quinolizinium compounds

After screening the peptide modification, we further explored the present reaction for protein modification by employing electron-deficient alkyne-containing quinoliziniums (Table 2). Bovine serum albumin (BSA; PDB ID: 4F5S) and human serum albumin (HSA; PDB ID: 1AO6) with a single free cysteine residue were utilized for bioconjugation. Treatment of BSA (0.1 mM) with quinolizinium reagent **1a** (1–2 equiv.) in 50 mM PBS (pH

Table 2 Bioconjugation reaction of quinolizinium compounds with proteins^a

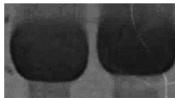
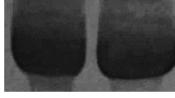
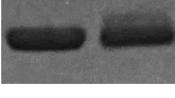
Entry	Protein	Quinolizinium	Conversion ^b (%)
1	BSA	1a	74 ^c
2	BSA	1a	80
3	BSA	1a	74 ^d
4	BSA	1b	65
5	BSA	1d	63
6	BSA	1e	28
7	BSA	1h	53
8	HSA	1a	70
9	HSA	1b	70
10	HSA	1d	75
11	HSA	1e	60
12	HSA	1h	59
13	Lysozyme	1a	ND
14	Lysozyme	1b	ND
15	Lysozyme	1d	ND
16	Lysozyme	1e	ND
17	Lysozyme	1h	ND

^a Treatment of proteins (0.1 mM) with quinolizinium (2 equiv.) in 50 mM PBS (pH 7.4)/DMSO (95:5) for 16 h. ^b Determined by LC-MS analysis; ND = not determined. ^c Quinolizinium (1 equiv.) was used. ^d 20 mM Tris·HCl (pH 7.4)/DMSO (95:5) was used.

7.4)/DMSO (95:5) at 25 °C for 16 h afforded **1a**-modified BSA in 74% and 80% conversions (entries 1 and 2), respectively, as confirmed by LC-MS analysis with other residues remained intact (Fig. S66 and S67, ESI†). In this regard, two equivalents of quinolizinium reagents were used for the bioconjugation of proteins as the unreacted reagents can be easily removed by size-exclusion chromatography (Bio-Rad Bio-Spin® 6). The reaction also worked well in 20 mM Tris·HCl (pH 7.4) (74%, entry 3; Fig. S68, ESI†).^{11a} Use of **1b**, **1d**, **1e** or **1h** (2 equiv.) afforded the corresponding modified BSA in 28–65% conversions (entries 4–7 and Fig. S69–S72, ESI†). Apart from BSA, HSA was also found to have 59–70% conversions (entries 8–12 and Fig. S77–S81, ESI†). In addition, trypsin digestion of modified BSA and HSA was conducted. After LC-MS/MS analysis of tryptic proteins, the modification was only found on Cys34 residue on peptide fragments, GLVLIAFSQYLQQCPFDEHVK of modified BSA and ALVLIAFAQYLQQCPFEDHVK of modified HSA, while other residues remained intact (Fig. S73–S75, S82 and S83, ESI†). Notably, no modification was observed with lysozyme (PDB ID: 3LYZ), which contains no free cysteine residue (Entries 13–17). These results indicated that our functionalized



Table 3 SDS-PAGE analysis of **1b**-modified proteins

Protein	1b			
	UV 472 nm		Coomassie blue	
	—	+	—	+
BSA				
HSA				
Lysozyme				

quinolizinium compounds could be employed as efficient chemoselective reagents in protein modification.

Labelling of proteins with fluorescent reagents can work as protein stains in SDS-PAGE gel and has the potential for *in vivo* tracking the uptake and physiological activities. Therefore, we performed the use of the present functionalized quinolizinium-based fluorescent reagents for labelling of proteins. As shown in SDS-PAGE analysis (Table 3), it was found that **1b**-modified BSA and HSA (from entries 4 and 9, Table 2) gave a fluorescent signal excitation at UV 472 nm in SDS-PAGE protein analysis. Treatment of **1b** with lysozyme gave no fluorescent signal (from entry 14, Table 2). The results of protein conjugates from SDS-PAGE analysis were found to be consistent to that of LC-MS analysis.

Biological studies of modified BCArg mutants

Human arginase is a metalloenzyme, in which its PEGylated form was the first generation of therapeutic protein with an extended half-life that has been reported to treat hepatocellular carcinoma,¹⁶ lung cancer,¹⁷ pancreatic cancer¹⁸ and immunotherapy-resistant melanoma¹⁹ in preclinical and clinical trials, respectively. Then, *Bacillus caldovelox* arginase mutant (BCArg mutant) and its PEGylated form (at the position

of Cys¹⁶¹) have been established as the second generation of therapeutic protein for the treatment of a broad spectrum of cancers such as lung and cervical cancers.²⁰ Apart from BSA and HSA, we also employed the use of present quinolizinium reagents to selectively modify cysteine residue of the BCArg mutant. BCArg mutant (0.1 mM) was first treated with quinolizinium **1a** (1 and 2 equiv.) in 20 mM Tris·HCl (pH 7.4)/DMSO (95 : 5) at 25 °C for 16 h to give **1a**-modified BCArg mutant in 60% and 82% conversions, respectively (Table 4; Fig. S85 and S86, ESI†). Then, **1b**, **1d**, **1e** and **1h** (2 equiv.) gave the corresponding modified BCArg mutants in 78–84% conversions (Table 4 and Fig. S87–S90, ESI†). Excellent cysteine selectivity was demonstrated by the tryptic peptide fragments of **1e**- and **1h**-modified BCArg mutants found in LC-MS/MS analysis (Fig. S91 and S92, ESI†).

To study the influence of the cationic quinolizinium-based reagents used in the modification on the biological properties of therapeutic proteins, we then evaluated enzyme activities and anti-cancer properties of the modified BCArg mutants with the unmodified analogue (Table 4). The enzymatic properties of the **1b**-, **1d**- and **1h**-modified BCArg mutants were comparable to that of the unmodified BCArg mutant, while **1a**- and **1e**-modified BCArg mutants displayed slightly lower enzymatic activities. The anti-cancer properties of the unmodified and modified BCArg mutants were then examined using human colon cancer cell line LoVo (CCL-229™, American Type Culture Collection). Experimental IC₅₀ values indicated that the anticancer efficacy of all modified BCArg mutants were comparable to that of the unmodified one. These findings indicated that the modified BCArg mutants retained their biological activities and anti-cancer efficacies after the bioconjugation with no significant influence over the cationic charge of the quinolizinium reagents.

Conclusions

In this work, we have developed a series of electron-deficient alkyne-containing quinoliziniums as useful reagents for cysteine-selective modification of peptides and proteins with up to 99% conversion. The present method was successfully applied on selective modification of proteins, including the therapeutic protein (BCArg mutant). Most of the resulting cationic quinolizinium-modified BCArg mutants have

Table 4 Enzymatic activities and IC₅₀ values of BCArg mutant and modified BCArg mutants

Sample ^a	Conversion ^b (%)	Specific activity (U mg ⁻¹)	IC ₅₀ values for LoVo (U mL ⁻¹)
BCArg mutant	—	202 ± 20	0.067 ± 0.012
1a -Modified BCArg mutant	60 ^c	—	—
1a -Modified BCArg mutant	82	138 ± 16	0.077 ± 0.025
1b -Modified BCArg mutant	80	179 ± 7	0.082 ± 0.030
1d -Modified BCArg mutant	84	185 ± 18	0.062 ± 0.008
1e -Modified BCArg mutant	78	130 ± 9	0.079 ± 0.022
1h -Modified BCArg mutant	80	169 ± 13	0.065 ± 0.004

^a Treatment of proteins (0.1 mM) with quinolizinium (2 equiv.) in 20 mM Tris·HCl (pH 7.4)/MSO (95 : 5) for 16 h. ^b Determined by LC-MS analysis.

^c Quinolizinium (1 equiv.) was used.

comparable enzymatic activities and anticancer efficacies to those of the unmodified one.

Experimental

All reagents were commercially available and used without further purification. All peptides (>98% purity) were directly purchased from GL Biochem (Shanghai) Ltd. BSA, HSA and lysozyme were purchased from Sigma Aldrich and used without further purification. Milli-Q® water used as reaction solvent in peptide and protein modification, as well as LC-MS analysis, was deionised using a Milli-Q® Gradient A10 system (Millipore, Billerica, USA). Flash column chromatography was performed using silica gel 60 (230–400 mesh ASTM) with ethyl acetate/n-hexane or methanol/dichloromethane as eluent. All NMR spectra were recorded on a Bruker DPX-400 spectrometer. All chemical shifts are quoted on the scale in ppm using TMS or residual solvent as the internal standard. Coupling constants (J) are reported in Hertz (Hz) with the following splitting abbreviations: s = singlet, br s = broad singlet, d = doublet, dd = double doublet, t = triplet and m = multiplet. All mass spectra were obtained on an ESI source of Agilent 6540 Ultra High Definition (UHD) Accurate-Mass Q-TOF LC/MS systems in the positive and negative ion modes.

All the photochemical experiments were performed in a custom made “light box” with 4 reaction vessels surrounded by 16 blue LED light bulbs. The temperature was maintained by a fan attached to the “light box”. A voltage transformer was connected with the blue LEDs and employed to monitor the power of the light source ($P = U \times I = 14.3 \text{ V} \times 2.3 \text{ A} = 32.9 \text{ W}$). 4 reactions were performed in the “light box” every time for measurement of the reaction yields. The emission spectra of the blue LEDs revealed a maximum emission wavelength of the light source at $\lambda_{\text{max}} = 468 \text{ nm}$ (Fig. S1, ESI†).

General procedure for visible light-mediated gold-catalyzed synthesis of quinolizinium compounds **1a–1h**

A solution of aryl diazonium salts **3a–3e** (1.2 equiv.), silyl substituted alkynes **2a** or **2b** (0.5 mmol, 1 equiv.), Ph_3PAuCl (10 mol%) and 5 mL of CH_3CN was added into a 20 mL test tube. The test tube capped with a rubber septum was evacuated and refilled with nitrogen three times. After that, the tube containing the reaction mixture was irradiated with blue LEDs for 16 h. After the reaction completed, the mixture was concentrated under reduced pressure. The crude product was purified by flash column chromatography using methanol/dichloromethane as eluent to afford compound **1a–1h**.

Compound 1a. Yellow powder, 49% yield. ^1H NMR (400 MHz, CD_3CN) δ 9.00 (d, $J = 9.0 \text{ Hz}$, 1H), 8.90 (t, $J = 7.7 \text{ Hz}$, 2H), 8.43 (d, $J = 8.2 \text{ Hz}$, 1H), 8.26–8.20 (m, 3H), 8.20–8.13 (m, 1H), 8.09–8.03 (m, 1H), 7.70 (t, $J = 7.5 \text{ Hz}$, 1H), 7.66 (d, $J = 8.4 \text{ Hz}$, 2H), 7.52 (d, $J = 8.9 \text{ Hz}$, 1H), 7.37–7.30 (m, 1H), 4.06 (s, 1H), 0.07 (d, $J = 3.3 \text{ Hz}$, 9H). ^{13}C NMR (100 MHz, CDCl_3) δ 176.23, 149.39, 144.58, 143.82, 143.11, 137.40, 136.77, 136.16, 135.28, 134.45, 132.40, 131.07, 130.71, 130.19, 129.75, 129.39, 129.16, 129.11, 128.35, 127.48, 125.31, 125.04, 119.24, 82.92, 79.92, 2.34. HRMS

(ESI) m/z calcd for $\text{C}_{29}\text{H}_{24}\text{NOSi}^+ [\text{M}-\text{BF}_4^-]^+$ 430.1622, found 430.1623; m/z calcd for $\text{BF}_4^- [\text{M}-\text{C}_{29}\text{H}_{24}\text{NOSi}^+]^-$ 87.0035, found 87.0034.

Compound 1b. Yellow powder, 25% yield. ^1H NMR (400 MHz, acetone- d_6) δ 9.35 (d, $J = 8.4 \text{ Hz}$, 1H), 9.27 (s, 1H), 8.59 (d, $J = 8.0 \text{ Hz}$, 1H), 8.35 (d, $J = 8.6 \text{ Hz}$, 2H), 8.34–8.25 (m, 1H), 8.21–8.12 (m, 2H), 8.07–7.99 (m, 4H), 7.90 (d, $J = 8.9 \text{ Hz}$, 1H), 7.83–7.75 (m, 4H), 7.56–7.47 (m, 1H), 4.61 (s, 1H), 0.17 (s, 9H). ^{13}C NMR (100 MHz, acetone- d_6) δ 196.12, 189.90, 184.16, 173.96, 168.85, 165.37, 157.32, 157.19, 157.03, 155.09, 154.50, 153.05, 150.89, 150.47, 150.22, 149.57, 149.45, 149.41, 149.35, 148.44, 148.25, 148.01, 147.73, 147.44, 146.32, 145.40, 138.26, 103.91, 99.90, 49.58, 49.39, 49.20, 49.00, 48.81, 48.62, 48.43, 21.34. HRMS (ESI) m/z calcd for $\text{C}_{35}\text{H}_{28}\text{NOSi}^+ [\text{M}-\text{BF}_4^-]^+$ 506.1935, found 506.1951; m/z calcd for $\text{BF}_4^- [\text{M}-\text{C}_{35}\text{H}_{28}\text{NOSi}^+]^-$ 87.0035, found 87.0034.

Compound 1c. Brown powder, 17% yield. ^1H NMR (400 MHz, CDCl_3) δ 9.00 (d, $J = 8.3 \text{ Hz}$, 1H), 8.93 (d, $J = 9.0 \text{ Hz}$, 1H), 8.75 (d, $J = 7.9 \text{ Hz}$, 1H), 8.27 (d, $J = 7.2 \text{ Hz}$, 2H), 8.07 (d, $J = 7.8 \text{ Hz}$, 1H), 7.66–7.54 (m, 5H), 7.44 (d, $J = 8.6 \text{ Hz}$, 1H), 7.24 (t, $J = 7.8 \text{ Hz}$, 1H), 4.12 (s, 3H), 3.62 (s, 1H), 0.13 (s, 9H). ^{13}C NMR (100 MHz, CDCl_3) δ 176.19, 164.59, 148.66, 145.13, 143.70, 141.75, 137.40, 135.98, 133.65, 132.36, 130.67, 130.19, 129.81, 129.57, 128.85, 128.38, 124.90, 124.10, 120.84, 119.57, 119.10, 111.28, 82.85, 79.92, 56.43, 31.56, 30.32, 2.40. HRMS (ESI) m/z calcd for $\text{C}_{30}\text{H}_{26}\text{NO}_2\text{Si}^+ [\text{M}-\text{BF}_4^-]^+$ 460.1727, found 460.1719; m/z calcd for $\text{BF}_4^- [\text{M}-\text{C}_{30}\text{H}_{26}\text{NO}_2\text{Si}^+]^-$ 87.0035, found 87.0033.

Compound 1d. Yellow powder, 41% yield. ^1H NMR (400 MHz, acetone- d_6) δ 10.19 (s, 1H), 9.29 (d, $J = 9.0 \text{ Hz}$, 1H), 9.12 (dd, $J = 13.1, 8.7 \text{ Hz}$, 2H), 8.53 (d, $J = 8.2 \text{ Hz}$, 1H), 8.38 (d, $J = 7.9 \text{ Hz}$, 1H), 8.25 (t, $J = 7.5 \text{ Hz}$, 1H), 8.11 (t, $J = 7.7 \text{ Hz}$, 1H), 7.90 (d, $J = 8.4 \text{ Hz}$, 2H), 7.85 (d, $J = 9.0 \text{ Hz}$, 1H), 7.79 (t, $J = 7.5 \text{ Hz}$, 1H), 7.65 (d, $J = 8.5 \text{ Hz}$, 2H), 7.50 (t, $J = 8.0 \text{ Hz}$, 1H), 3.85 (s, 1H), 0.16 (s, 9H). ^{13}C NMR (100 MHz, acetone- d_6) δ 169.64, 166.67, 162.03, 160.83, 157.29, 156.92, 154.73, 154.39, 153.82, 152.95, 150.28, 149.70, 149.33, 149.21, 149.17, 149.11, 147.04, 145.60, 145.36, 139.99, 139.92, 138.83, 49.58, 49.39, 49.20, 49.00, 48.81, 48.62, 48.43, 21.39. HRMS (ESI) m/z calcd for $\text{C}_{29}\text{H}_{25}\text{N}_2\text{OSi}^+ [\text{M}-\text{BF}_4^-]^+$ 445.1731, found 445.1790; m/z calcd for $\text{BF}_4^- [\text{M}-\text{C}_{29}\text{H}_{25}\text{N}_2\text{OSi}^+]^-$ 87.0035, found 87.0032.

Compound 1e. Deep yellow powder, 45% yield. ^1H NMR (400 MHz, CD_3CN) δ 9.19 (s, 1H), 8.96 (d, $J = 8.5 \text{ Hz}$, 1H), 8.89 (s, 1H), 8.38 (d, $J = 8.3 \text{ Hz}$, 1H), 8.12 (t, $J = 7.5 \text{ Hz}$, 1H), 8.06 (d, $J = 7.5 \text{ Hz}$, 1H), 8.00 (t, $J = 7.5 \text{ Hz}$, 1H), 7.92 (dd, $J = 6.4, 2.9 \text{ Hz}$, 2H), 7.77–7.68 (m, 6H), 7.62 (t, $J = 7.6 \text{ Hz}$, 1H), 7.50 (d, $J = 8.0 \text{ Hz}$, 2H), 7.35 (t, $J = 8.6 \text{ Hz}$, 1H), 3.45 (s, 1H), 0.10 (s, 9H). ^{13}C NMR (100 MHz, acetone- d_6) δ 173.53, 168.68, 157.42, 157.30, 155.12, 154.32, 153.32, 153.00, 150.76, 150.20, 150.08, 149.29, 149.21, 149.11, 148.02, 147.54, 147.33, 146.11, 145.28, 139.94, 138.18, 95.63, 49.58, 49.39, 49.19, 49.00, 48.81, 48.62, 48.42, 21.37. HRMS (ESI) m/z calcd for $\text{C}_{35}\text{H}_{29}\text{N}_2\text{OSi}^+ [\text{M}-\text{BF}_4^-]^+$ 521.2044, found 521.2052; m/z calcd for $\text{BF}_4^- [\text{M}-\text{C}_{35}\text{H}_{29}\text{N}_2\text{OSi}^+]^-$ 87.0035, found 87.0032.

Compound 1f. Yellow powder, 48% yield. ^1H NMR (400 MHz, CD_3CN) δ 9.23 (s, 1H), 8.81–8.74 (m, 2H), 8.66 (d, $J = 9.0 \text{ Hz}$, 1H), 8.11 (d, $J = 7.8 \text{ Hz}$, 1H), 7.69 (d, $J = 8.5 \text{ Hz}$, 2H), 7.65–7.60 (m, 2H), 7.59 (d, $J = 2.2 \text{ Hz}$, 1H), 7.50 (d, $J = 8.9 \text{ Hz}$, 1H), 7.40 (d, $J =$



8.5 Hz, 2H), 7.30 (dd, J = 11.7, 4.3 Hz, 1H), 4.11 (s, 3H), 3.45 (s, 1H), 0.10 (s, 9H). ^{13}C NMR (100 MHz, CD_3OD) δ 165.86, 152.41, 150.02, 148.25, 141.86, 137.87, 135.94, 134.02, 133.64, 130.60, 130.47, 130.08, 129.70, 126.39, 121.75, 121.40, 120.74, 119.42, 111.51, 77.20, 56.96, 2.28. HRMS (ESI) m/z calcd for $\text{C}_{30}\text{H}_{27}\text{N}_2\text{O}_2\text{Si}^+ [\text{M}-\text{BF}_4^-]^+$ 475.1836, found 475.1847; m/z calcd for $\text{BF}_4^- [\text{M}-\text{C}_{30}\text{H}_{27}\text{N}_2\text{OSi}^+]^-$ 87.0035, found 87.0034.

Compound 1g. Yellow powder, 43% yield. ^1H NMR (400 MHz, CD_3OD) δ 9.24 (d, J = 9.0 Hz, 2H), 9.08 (d, J = 8.9 Hz, 1H), 8.58 (s, 1H), 8.30 (dd, J = 19.0, 8.2 Hz, 2H), 7.78 (dd, J = 12.3, 5.7 Hz, 4H), 7.54–7.42 (m, 3H), 3.85 (s, 1H), 0.16 (s, 9H). ^{13}C NMR (100 MHz, CD_3OD) δ 144.33, 138.08, 136.32, 133.96, 133.12, 131.30, 130.70, 130.36, 129.92, 126.62, 125.50, 121.46, 119.88, 49.64, 49.43, 49.21, 49.00, 48.79, 48.57, 48.36, 2.17. ^{19}F NMR (376 MHz, DMSO-d_6) δ -61.80, -148.29. HRMS (ESI) m/z calcd for $\text{C}_{30}\text{H}_{24}\text{F}_3\text{N}_2\text{OSi}^+ [\text{M}-\text{BF}_4^-]^+$ 513.1605, found 513.1616; m/z calcd for $\text{BF}_4^- [\text{M}-\text{C}_{30}\text{H}_{24}\text{F}_3\text{N}_2\text{OSi}^+]^-$ 87.0035, found 87.0036.

Compound 1h. Yellow powder, 10% yield. ^1H NMR (400 MHz, CD_3OD) δ 9.12 (d, J = 8.5 Hz, 1H), 9.05 (d, J = 8.4 Hz, 1H), 8.60 (d, J = 8.3 Hz, 1H), 8.36 (d, J = 7.4 Hz, 1H), 8.31 (d, J = 7.8 Hz, 1H), 8.22 (dd, J = 15.7, 7.6 Hz, 2H), 8.16 (dd, J = 18.0, 9.7 Hz, 1H), 8.11 (d, J = 7.4 Hz, 1H), 8.07 (t, J = 7.8 Hz, 1H), 7.96 (d, J = 8.5 Hz, 2H), 7.62 (d, J = 8.6 Hz, 2H), 3.86 (s, 1H), 0.22 (s, 9H). ^{13}C NMR (100 MHz, CD_3OD) δ 152.55, 150.28, 145.69, 142.47, 139.33, 136.23, 134.96, 134.43, 133.79, 133.42, 131.96, 131.06, 130.88, 129.47, 128.55, 126.13, 122.67, 121.99, 77.29, 49.64, 49.43, 49.22, 49.00, 48.79, 48.58, 48.37, 1.69. HRMS (ESI) m/z calcd for $\text{C}_{29}\text{H}_{25}\text{N}_2\text{OSi}^+ [\text{M}-\text{BF}_4^-]^+$ 445.1731, found 445.1741; m/z calcd for $\text{BF}_4^- [\text{M}-\text{C}_{29}\text{H}_{25}\text{N}_2\text{OSi}^+]^-$ 87.0035, found 87.0033.

Author contributions

M.-K. Wong and Y.-C. Leung conceptualized and supervised the study. C.-F. Xu and K. K.-Y. Kung performed the organic synthesis in this work. C.-F. Xu, K. K.-Y. Kung and Q. Yu performed characterization of all compounds. K. K.-Y. Kung and W.-Y. O performed UV/Vis and fluorescence measurements, bioconjugation experiments and mass spectroscopy analysis of this work. S.-F. Chung and S.-Y. Tam purified and measured the biological activities of the anticancer proteins. K. K.-Y. Kung prepared this paper.

Conflicts of interest

M.-K. Wong, K. K.-Y. Kung, Q. Yu and W.-Y. O filed a patent on electron-deficient alkyne-containing quinolizinium compounds (CN113402538A).

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