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ARTICLE

Unique Photophysical Properties of 9-Styryl-1,2-dihydropyrrolo[3,4-*β*]indolizin-3-one and Their Efficient Synthesis via Direct C-H Activation†

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Styryl Seoul-Fluor (SF) skeleton was rationally designed by introducing olefin unit at the C-9 of 1,2-dihydropyrrolo[3,4-*β*]indolizin-3-one via regioselective direct C–H activation. We synthesized a series of styryl SF analogues, maintaining unique photophysical properties that can be systematically controlled by electronic characters of the substituents, with an average bathochromic shift of 39 nm in emission maxima.

Introduction

Fluorescent organic molecules have received significant attention as research tools in biological science and clinical diagnosis due to their excellent selectivity, sensitivity, large linear range of analysis, and ease of handling.[1] Despite the high demand for fluorescent materials, only a limited number of chemical families are routinely used since the development of novel fluorophore scaffolds is still challenging.[2] To obtain the desired photophysical properties, recent studies have been focused on the structural modification of existing fluorophores. The following strategies have been used to study the structure-photophysical property relationship (SPPR) on a certain fluorophore: 1) introduction of electron-donating or withdrawing groups,[3] 2) rigidification of rotatable moieties,[4] 3) replacement of a carbon or oxygen atom with a heteroatom,[5] 4) extension of the π-conjugated system,[6] and 5) fusion of a heteroaromatic ring onto fluorophores.[7]

Among these approaches, extension of the π-conjugated system via the insertion of an additional double bond into the organic fluorophores has been widely used to modulate their photophysical properties. In the case of cyanine dye, the addition of an olefin unit in the linker region between two indoles results in a bathochromic shift of 100 nm in its emission maxima (Fig. 1).[8] Further extension of the π conjugation with an additional olefin unit led to another 100-nm red shift to the near infrared region. An identical strategy was applied to BODIPY TR (left) to achieve a bathochromic shift of 32 nm in BODIPY 630/650 (right) via olefin insertion (Fig. 1).[9] In the case of the coumarin-derived dye-sensitized solar cell (DSSC), an addition of olefin unit between the cyanoacrylic acid and the coumarin core produced two-fold increase in absorption bandwidth in the visible region as well as a red shift in absorption maxima, which led to a significant improvement in the light-harvesting ability of DSSC dyes as photosensitizers.[10]

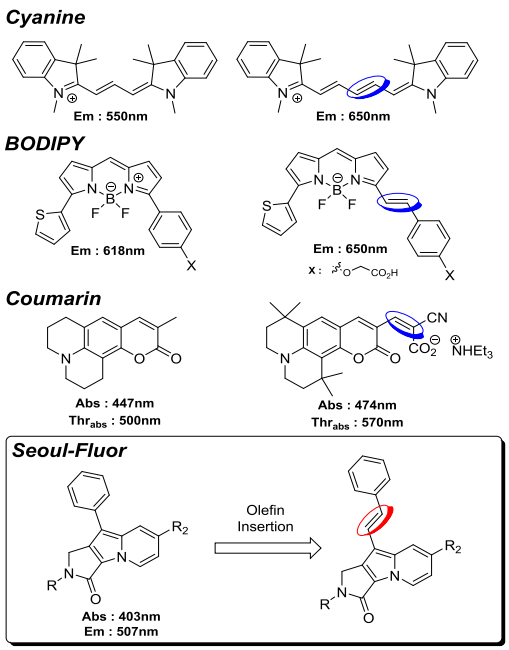


Fig. 1 Influence of an additional olefin on the emission maxima for fluorescent molecules and absorption threshold/maxima of DSSC dye.

We reported the discovery of 1*H*-pyrrolo-[3,4-*β*]indolizin-3-one, a full-color-tunable fluorophore, named as Seoul-Fluor (SF).[11] We also demonstrated the predictability of its emission maximum through a systematic correlation study of the electronic properties of substituents at the C-7 and C-9 positions of SF.[12] Application of SF as a platform for fluorescent bioprobes allowed the real-time monitoring of pH,[13] VHR phosphatase activity,[14] cellular lipid droplet,[15] etc. In

addition to emission wavelength, we systematically controlled the fluorescence quantum yields through the regulation of photoinduced electron transfer (PeT) within the core skeletons. Development of a facile synthetic method to produce SF using direct C–H activation allowed access to a broad substrate scope for various SF analogues.[16] Overall strategy about the discovery of SF, fundamental understanding of its structure-photophysical property relationship, and its subsequent biological application was recently reported as an account.[17]

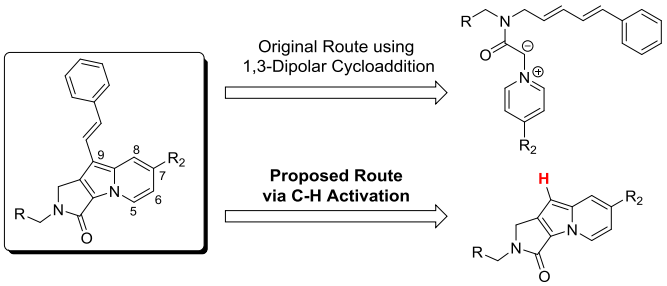


Fig. 2 Original and proposed synthetic routes for the olefin insertion at the C-9 position of Seoul-Fluor.

With a novel fluorescent core in hand, we envisioned further diversification of Seoul-Fluor's photophysical properties via olefin insertion. Through the calculation of atomic coefficients of highest occupied molecular orbital (HOMO) and lowest occupied molecular orbital (LUMO) (Fig. S1), we determined the C-9 position as the ideal site for substituents to maximize the systematic perturbation of their photophysical properties. Previous observation also indicated that substituents with different electronic properties at the C-9 position caused dramatic changes in the emission wavelength.[12] Therefore, we aimed to explore a new version of Seoul-Fluor, namely styryl SF, via olefin insertion at the C-9 position. We envisioned that the extension of the π-conjugated system would lead to a diversification of their photophysical properties. As shown in Fig. 2 (upper), insertion of an olefin between the γ-lactam-embedded indolizine core and aryl ring might be achieved through intramolecular 1,3-dipolar cycloaddition of β,γ,δ,ε-unsaturated amide with a pendant azomethine ylide. Unfortunately, we failed to obtain the olefin-inserted SF product even after extensive trials, which is probably due to the low reactivity of the dipolarophile, β,γ-unsaturated carbon, in the presence of δ,ε-unsaturated styryl group acting as an electron sink. To overcome this synthetic challenge, we aimed to develop an efficient synthetic route for the olefin insertion in Seoul-Fluor framework. As shown in Fig. 2 (lower), disconnection of the styryl group at the C-9 position allows construction via a modular strategy for efficient diversification at the late-stage of the synthesis. Therefore, we applied this new synthetic route to introduce styryl units into the preformed indolizine core using a regioselective Heck-type coupling of substituted styrenes via direct C–H activation at the C-9 position of 1*H*-pyrrolo-[3,4-*β*]indolizin-3-one.[16]

Results and discussion

1,3-Dipolar cycloaddition of the terminal acetylene as a robust synthesis of the indolizine-based core.

We initiated our studies by synthesizing the γ-lactam-embedded indolizines **4a**–**c**, the key substrates for direct C–H activation. As shown in Fig. 3, secondary amine **2** was prepared by nucleophilic substitution of propargyl amine with Boc-protected 3-aminopropyl bromide **1**. The resulting amine **2** was acylated with 2-bromoacetyl

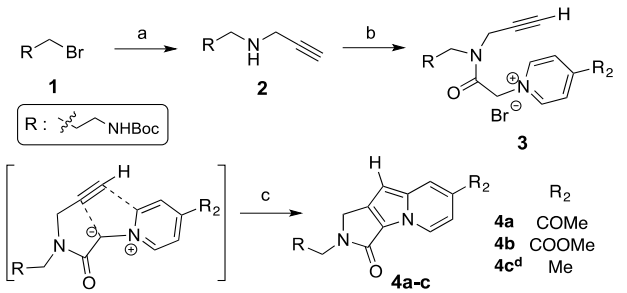


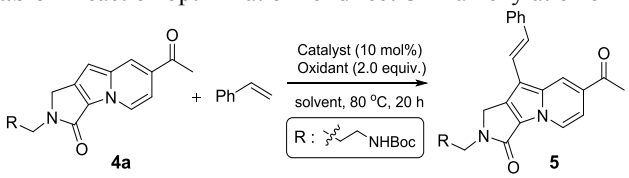
Fig. 3 Synthesis of **4a**–**c**. Reagents and conditions: (a) propargyl amine, DIPEA, ACN, 60 °C; (b) bromoacetyl bromide, TEA, DCM, –78 °C; then 4-substituted pyridine, DCM, 60 °C; (c) DBU, toluene/DCM (1:1, v/v); (d) Ag_2O was added during the cycloaddition reaction.

bromide, followed by the treatment of substituted pyridines to afford the pyridinium salts **3**. The resulting pyridinium intermediate **3** was subjected, without further purification, to intramolecular [3+2] cycloaddition with the pendant terminal acetylene. The azomethine ylide was generated *in-situ* upon treatment with 1,8-diazabicyclo [5.4.0]undec-7-ene (DBU). Unlike the alkenyl dipolarophiles in our original procedure,[11,12] intramolecular 1,3-dipolar cycloaddition of alkynyl dipolarophiles allows spontaneous oxidative aromatizaton of the resulting tricyclic adduct to afford γ-lactam-embedded indolizines **4a**–**c** in good to moderate yields, without the need for additional oxidants such as 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ). In the case of **4c**, containing an electron-donating methyl group as the R_2 substituent, we observed an improved yield for the 1,3-dipolar cycloaddition upon activation of the alkyne in the presence of silver oxide.[16,18]

Regioselective Pd-mediated C–H activation for introduction of styryl moiety to indolizine core.

We then screened different reaction conditions for palladium-mediated C–H activation of the C-9 position using **4a** as a model substrate. As shown in Table 1, styrene was employed as the model olefin, for simplicity. The initial reaction was not catalytic in

Table 1 Reaction optimization for direct C–H alkenylation of **4a**.

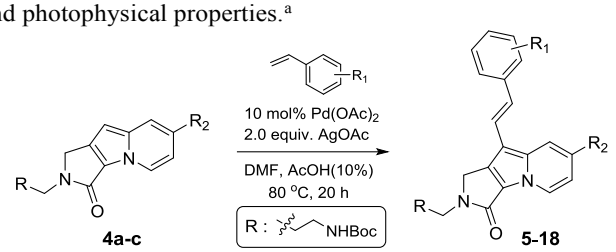


Entry	Catalyst	Solvent	Oxidant	Yield[a]
1	$PdCl_2$	DMF	-	4.0
2	$Pd(PPh_3)_2Cl_2$	DMF	-	0.3
3	$Pd(OAc)_2$	DMF	-	5.7
4	$Pd(OAc)_2$	DMF	DDQ	6.7
5	$Pd(OAc)_2$	DMF	H_2O_2	17
6	$Pd(OAc)_2$	DMF	$Cu(OAc)_2$	7.2
7	$Pd(OAc)_2$	DMF	AgOAc	74
8	$Pd(OAc)_2$	DMSO	AgOAc	63
9	$Pd(OAc)_2$	dioxane	AgOAc	17
10	$Pd(OAc)_2$	NMP	AgOAc	89
11	$Pd(OAc)_2$	NMP, AcOH (10%)	AgOAc	74
12	$Pd(OAc)_2$	DMF, AcOH (10%)	AgOAc	95

[a]Determined by HPLC analysis using 7-hydroxycoumarin as an internal standard.

palladium and the desired product was formed in low yield in the presence of a Pd (II) catalyst (Table 1, Entries 1–3). Excellent regioselectivity was observed for the functionalization of the C-9 position over the C-6 position of the indolizine core. In contrast to the Pd(II) catalysts used, Pd(0) catalysts including $Pd(PPh_3)_4$ and $Pd_2(dba)_3$ provided none of the desired product **5** (data not shown). Based on this observation, we hypothesized that regeneration of Pd(II) from Pd(0) through the addition of a stoichiometric oxidant would result in increasing catalytic turnover. With this initial result in hand, we optimized the reaction conditions for **4a** using $Pd(OAc)_2$ and screened various oxidants, solvents, and temperatures. Among the oxidants screened, AgOAc was the most effective in increasing the catalytic turnover rate for this transformation (Table 1, Entries 4–7). After a solvent screening (Table 1, Entries 7–12), we finalized the optimal conditions for site-specific alkenylation via direct C–H activation in the presence of 10mol% $Pd(OAc)_2$ and stoichiometric AgOAc under the elevated temperature. Further optimization indicated that the addition of 10% acetic acid as a co-solvent resulted in an excellent yield (Table 1, Entry 12). This result is presumably due to the role of the acetate ion as a base/ligand, which is in accordance with a concerted metalation deprotonation (CMD) pathway.[19]

Table 2 Electronic effects of substituents on the isolated yield and photophysical properties.[a]



Prod	R_1	R_2	Yield	λ_{abs}[c]	λ_{em}[d]	Stokes shift	Φ_F[e]	ε[f]
6	4-NO_2	Ac	83[b]	450	618	168	0.40	2.9E4
7	F_5	Ac	99	415	527	112	0.42	2.2E4
8	4-CF_3	Ac	76	428	533	105	0.35	1.7E4
9	4-Br	Ac	98	433	545	112	0.29	1.7E4
5	H	Ac	94	424	539	115	0.30	1.4E4
10	4-Me	Ac	42	428	545	117	0.21	1.4E4
11	4-OMe	Ac	49	433	557	124	0.10	1.3E4
12[g]	4-NH_2	Ac	51	450	588	138	0.10	8.6E3
13	4-Br	CO_2Me	85	409	529	120	0.21	3.5E3
14	H	CO_2Me	64	409	522	113	0.21	2.4E3
15	4-OMe	CO_2Me	75	413	534	121	0.16	9.3E2
16	4-Br	Me	25	349	460	111	0.12	1.1E4
17[h]	H	Me	20	350	480	130	0.09	1.3E4
18	4-OMe	Me	22	340	431	91	0.07	1.8E3

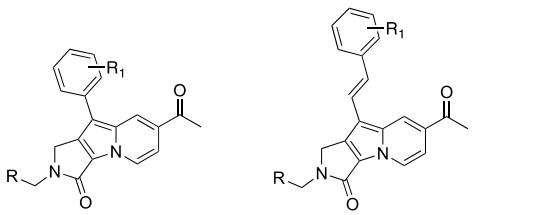
[a]See the Supporting Information for detailed experimental procedures. [b]Recrystalization yield. [c]Longest absorption wavelength maxima. [d]Excited at the longest absorption wavelength maxima. [e]Absolute fluorescence quantum yield. [f]Molar extinction coefficient. [g]Obtained via the reduction of **6**. [h]$Cu(OAc)_2$ was used as the oxidant

Examination of the substrate scope for this transformation was undertaken by reacting **4a** with various substituted styrenes containing both electron-withdrawing (NO_2, F_5, CF_3, Br) and electron-donating functional groups (Me, OMe, NH_2). As shown in Table 2, all styryl SF analogues (**5**–**12**) were obtained in moderate to excellent yields. No regioisomers were observed for any of the styrene derivatives, further confirming the excellent regioselectivity of this direct C–H activation. In addition to acetyl indolizine **4a**, the coupling efficiency of indolizine cores bearing both an electron-withdrawing methyl ester (**4b**) and an electron-donating methyl group (**4c**) were examined. Reactivity of **4b** was comparable to **4a** and afforded olefinated products with various substituents (**13**–**15**) in moderate yields. Methyl indolizine **4c**, however, produced significantly diminished yields for the Heck-type coupling product (**16**–**18**), which is consistent with our previous observations.[16] In the case of **17**, copper acetate was a more effective oxidant than silver acetate. It is worth mentioning that the regioselective olefination of **4a**–**c** by this method allows the late-stage functionalization, which ensures the efficient construction of styryl SF analogues with excellent diversity.

General bathochromic shift in emission wavelength of styryl Seoul-Fluor analogs.

Having established a new synthetic method to access the styryl SF analogues, we immediately turned our attention to the analysis of their photophysical properties including absorption/emission maxima ($\lambda_{abs}/\lambda_{em}$), quantum yield ($\Phi_F$), and molar extinction coefficient (ε). As previously proposed, extension of the π-conjugated system via the insertion of an additional double bond in Seoul-Fluor might lead to the perturbation of their photophysical properties. Compared to the original SF series, styryl SF analogues showed a decrease in quantum yields, potentially due to the increased non-radiative energy loss associated with additional rotatable bonds at the C-9 position (Table 3). However, the quantum yield is elaborately controlled by the electronic characters of the C-9 substituents, consistent with our previous report.[16]

Table 3. Direct comparison of molecular structures and photophysical properties of Seoul-Fluor and styryl Seoul-Fluor analogues.[a]



λ_{abs}[b]	λ_{em}[c]	Φ_F[d]	R_1	λ_{abs}[b]	λ_{em}[c]	Φ_F[d]
386	463	0.91	2,3,4,5,6-F_5	415	527	0.42
400	496	0.90	4-CF_3	428	533	0.35
405	502	0.85	4-Br	433	545	0.29
403	507	0.99	H	424	539	0.30
408	509	0.71	4-Me	428	545	0.21
412	526	0.62	4-OMe	433	557	0.10
416	558	0.22	4-NH_2	450	588	0.10

[a]Photophysical properties of all samples were measured in dichloromethane. [b]Longest absorption wavelength maxima. [c]Excited at the longest absorption wavelength maxima. [d]Absolute fluorescence quantum yield.

A bathochromic shift in emission wavelength was observed for all styryl SF analogues when compared to the original SF series. Increasing the electron-donating ability of the R_1 substituent in styryl SF's from F_5 (**7**) to NH_2 (**12**) produced a bathochromic shift in emission wavelength from 527 nm to 588 nm. In accordance with our hypothesis, we observed a general bathochromic shift in emission wavelength through the olefin insertion to Seoul-Fluor. Emission wavelength of styryl SF with NO_2 (**6**) shows significantly red-shifted emission wavelength (618 nm) compared to other styryl SF analogues.

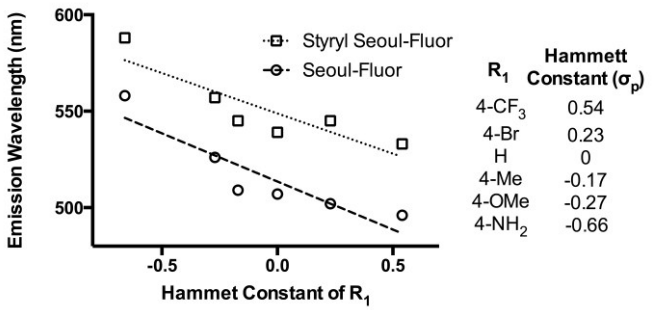


Fig. 4 Correlation plot between emission wavelength and Hammett constant (σ_p) of R_1 substituents in original SF and styryl SF analogues.

This phenomenon was presumably due to the characteristics of nitro group via n→π* transition and the detailed influence of nitro group on the photophysical properties of styryl SF will be reported in due course. As shown in Table 3, the emission wavelength of styryl SF analogues showed an average of 39 nm of bathochromic shift compared to that of original SFs. The emission wavelength shift produced by olefin insertion in Seoul-Fluor is not as drastic as that observed in the cyanine case. This might be caused by the distortion of bond angles between the indolizine ring and styryl group leading to the reduction of efficiency in the π conjugation system.

Since it is reasonable to infer that the electronic perturbation produced by the styryl substituents influences the photophysical properties of the styryl SF analogues, we plotted the emission wavelength versus the Hammett constant (σ_p), which represents the electronic properties of the R_1 substituents (Fig. 4).[20] Both SF and styryl SF analogues showed a positive correlation with emission wavelength and an inverse correlation with quantum yields (Fig. S2) in proportion to the electron donating ability of the R_1 group. Therefore, the new styryl SFs are expected to serve as a discovery platform for novel bioprobes with longer emission wavelengths, while maintaining the advantageous tunability of SFs in emission wavelength and quantum yields.

Conclusions

We have developed a novel palladium-catalyzed regioselective olefination of 1,2-dihydropyrrolo[3,4-*β*]indolizin-3-one, namely Seoul-Fluor, via direct C–H activation to overcome limitations with the existing synthetic process for styryl Seoul-Fluor analogues. With this new synthetic route in hand, we could efficiently access a diverse collection of styryl SF analogues with excellent substrate generality via the late-stage diversification. In agreement with our hypothesis, we observed the general bathochromic shifts of emission wavelength maxima in styryl SF analogues (39 nm in average), compared to that of SF analogues. This result confirms that the extension of π-conjugated system by olefin insertion between indolizine core and C-9 substituents in Seoul-Fluor leads to the perturbation of its photophysical properties. This palladium-mediated site-specific olefination via direct C–H activation is a powerful and atom-economic method for the olefination of heterocyclic cores, which allows for the efficient diversification of Seoul-Fluor at the late stage of the synthesis.

Experimental

General Methods

^{1}H and ^{13}C NMR spectra were recorded on Bruker DRX-300 (Bruker Biospin, Germany) and Varian Inova-500 (Varian Assoc, Palo Alto, USA), chemical shifts were measured in ppm downfield from internal tetramethylsilane (TMS) standard. Multiplicity was indicated as follows: s (singlet); d (doublet); t (triplet); q (quartet); m (multiplet); dd (doublet of doublet); dt (doublet of triplet); br s (broad singlet), etc. High resolution mass analyses were performed at the Mass Spectrometry Laboratory of Seoul National University by direct injection on a JEOL JMS AX505WA spectrometer using fast atom bombardment (FAB) method. Absorbance of final fluorescence compounds was measured by UV-VIS spectrophotometer UV-1650PC (Shimatzu, Japan). Excitation and Emission maxima were measured by Cary Eclipse Fluorescence spectrophotometer (Varian Assoc., Palo Alto, USA). Absolute quantum yield was measured by absolute PL quantum yield measurement system QE-1000 (OTSUKA Electronics). *In silico* calculations were performed using the Materials Studio® 4.2 program (Accelrys Software Inc.) A generalized gradient approximation (GAA) for the exchange correlation function of Perdew, Burke, and Ernzerhof (PBE) was used with the double numerical basis set with polarization (DNP) as implemented in DMol3. Internal standard analysis for checking yield of crude reaction was performed on SHIMADZU HPLC equipped with a reverse phase column (XDB C18, 5 μm, 4.6 × 150 mm). Samples were analyzed starting with 5% ACN in H_2O (0.1% TFA) for 5 min after injected 10 μL of sample and solvent was changed from 5% ACN in H_2O (0.1% TFA) to 100% ACN (0.1% TFA) for 30 min with 1.0 mL/min flow. Absorbance was detected by 365 nm.

Materials

Chemical reagents and solvents were purchased either from Sigma-Aldrich and Tokyo Chemical Industry Co., or Acros, and used without further purification. **1** and **4a**–**c** were prepared with previously reported protocols.[16]

General procedure for cross-coupling reactions

To a solution of γ-lactam embedded indolizines (**4a**–**c**) in dimethylformamide, 10 volume% acetic acid, styrene derivatives (3 equiv.), palladium acetate (0.1 equiv.) and silver acetate (2 equiv.) were added and stirred at 80 °C for overnight (20 h). After the reaction completion as monitored by TLC, reaction mixture was concentrated *in vacuo* after filtration. The residue was purified by silica gel flash column chromatography to afford the desired product (**5**–**18**).

***tert*-Butyl (E)-(3-(7-acetyl-3-oxo-9-styryl-1,3-dihydro-2H-pyrrolo [3,4-*β*]indolizin-2-yl)propyl)carbamate (5).** ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.49 (d, *J* = 7.2 Hz, 1H), 8.30 (s, 1H), 7.55 (d, *J* = 7.8 Hz, 2H), 7.43-7.32 (m, 4H), 7.25 (d, *J* = 7.2 Hz, 1H), 6.69 (d, *J* = 15.9 Hz, 1H), 5.36 (br s, 1H), 4.57 (s, 2H), 3.73 (t, *J* = 6.0 Hz, 2H), 3.21 (d, *J* = 5.1 Hz, 2H), 2.68 (s, 3H), 1.92–1.88 (m, 2H), 1.46 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 193.4, 161.7, 156.2, 137.3, 136.0, 133.1, 128.8, 128.7, 128.5, 127.9, 127.5, 126.0, 124.6, 123.4, 120.0, 117.8, 112.0, 109.7, 79.2, 47.2, 40.2, 37.4, 28.8, 28.4, 26.2; HRMS (FAB+) m/z calcd. for $C_{28}H_{31}N_3O_4$ $[M]^+$ 473.23; found: 473.2315.

***tert*-Butyl (E)-(3-(7-acetyl-9-(4-nitrostyryl)-3-oxo-1,3-dihydro-2H-pyrrolo[3,4-*β*]indolizin-2-yl)propyl)carbamate (6).** ^{1}H NMR (300 MHz, DMSO) δ 8.79 (s, 1H) 8.42 (d, *J* = 7.2 Hz, 1H), 8.21 (d, *J* = 8.7 Hz, 2H), 8.05 (d, *J* = 15.9 Hz, 1H), 7.89 (d, *J* = 8.7 Hz, 2H), 7.24 (d, *J* = 7.2 Hz, 1H), 6.79 (d, *J* = 16.2 Hz, 1H), 4.69 (s, 2H), 3.51 (t, *J* = 6.6 Hz, 2H), 3.01 (dd, *J* = 11.0, 5.0 Hz, 2H), 2.66 (s, 3H), 1.81–1.76

(m, 2H), 1.38 (s, 9H); HRMS (FAB+) m/z calcd. for $C_{28}H_{30}N_4O_6$ $[M]^+$ 518.22; found: 518.2165.

***tert*-Butyl (*E*)-(3-(7-acetyl-3-oxo-9-(2-(perfluorophenyl)vinyl)-1,3-dihydro-2*H*-pyrrolo[3,4-*β*]indolizin-2-yl)propyl)carbamate (7).** ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.56 (d, *J* = 7.2 Hz, 1H), 8.27 (s, 1H), 7.73 (d, *J* = 16.5 Hz, 1H), 7.33 (d, *J* = 7.2 Hz, 1H), 6.51 (d, *J* = 16.5 Hz, 1H), 5.30 (br s, 1H), 4.59 (s, 2H), 3.74 (t, *J* = 6.5 Hz, 2H), 3.21 (dd, *J* = 12.3, 6.0 Hz, 2H), 2.69 (s, 3H), 1.95–1.86 (m, 2H), 1.45 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 195.2, 161.4, 156.1, 136.8, 133.6, 129.8, 126.3 (t, $^2J_{C,F}$ = 9.4 Hz), 126.2, 124.9, 124.0, 119.5, 112.6, 112.5, 111.4, 111.38, 111.33, 111.2, 110.2, 79.2, 47.0, 40.3, 37.3, 29.7, 28.8, 28.4, 26.2; HRMS (FAB+) m/z calcd. for $C_{28}H_{26}F_5N_3O_4$ $[M+H]^+$ 564.19; found: 564.1922.

***tert*-Butyl (*E*)-(3-(7-acetyl-3-oxo-9-(4-(trifluoromethyl)styryl)-1,3-dihydro-2*H*-pyrrolo[3,4-*β*]indolizin-2-yl)propyl)carbamate (8).** ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.52 (d, *J* = 7.2 Hz, 1H), δ 8.32 (s, 1H), 7.64 (s, 4H), 7.48 (d, *J* = 16.2 Hz, 1H), 7.29 (d, *J* = 7.2 Hz, 1H), 6.69 (d, *J* = 16.2 Hz, 1H), 5.31 (br s, 1H), 4.60 (s, 2H), 3.73 (t, *J* = 6.3 Hz, 2H), 3.21 (dd, *J* = 12.3, 6.0 Hz, 2H), 2.69 (s, 3H), 1.95–1.86 (m, 2H), 1.45 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 195.3, 161.5, 156.1, 140.8, 139.8, 136.4, 133.5, 129.2, 129.0 (q, $^2J_{C,F}$ = 31.5 Hz), 126.0, 125.7 (q, $^3J_{C,F}$ = 3.8 Hz), 125.7 (q, $^1J_{C,F}$ = 143 Hz), 123.7, 120.3, 119.6, 111.3, 110.0, 79.2, 47.1, 40.2, 37.4, 29.7, 28.8, 28.4, 26.3; HRMS (FAB+) m/z calcd. for $C_{29}H_{30}F_3N_3O_4$ $[M]^+$ 541.22; found: 541.2188.

***tert*-Butyl (*E*)-(3-(7-acetyl-9-(4-bromostyryl)-3-oxo-1,3-dihydro-2*H*-pyrrolo[3,4-*β*]indolizin-2-yl)propyl)carbamate (9).** ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.52 (d, *J* = 6.6 Hz, 1H), 8.31 (s, 1H), 7.52 (d, *J* = 8.4 Hz, 2H), 7.42 (d, *J* = 7.5 Hz, 2H), 7.36 (s, 1H), 7.27 (s, 1H), 6.62(d, *J* = 15.9 Hz, 1H), 5.32 (br s, 1H), 4.58 (s, 2H), 3.75–3.65 (m, 2H), 3.21 (dd, *J* = 11.4, 5.7 Hz, 2H), 2.69 (s, 3H), 1.92–1.82 (m, 2H), 1.45 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 195.3, 161.6, 156.1, 138.0, 136.3, 133.2, 131.9, 128.9, 127.4, 127.1, 124.7, 123.6, 122.1, 121.1, 119.8, 118.6, 111.6, 109.8, 79.2, 47.1, 40.2, 37.4, 29.7, 28.4, 26.3; HRMS (FAB+) m/z calcd. for $C_{28}H_{30}BrN_3O_4$ $[M]^+$ 551.14; found: 551.1420.

***tert*-Butyl (*E*)-(3-(7-acetyl-9-(4-methylstyryl)-3-oxo-1,3-dihydro-2*H*-pyrrolo[3,4-*β*]indolizin-2-yl)propyl)carbamate (10).** ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.46 (d, *J* = 7.8 Hz, 1H), 8.28 (s, 1H), 7.44 (d, *J* = 7.8 Hz, 2H), 7.34 (s, 1H), 7.24–7.19 (m, 3H), 6.65 (d, *J* = 15.9 Hz, 1H), 5.36 (br s, 1H), 4.55 (s, 2H), 3.71 (t, *J*= 6.5 Hz, 2H), 3.20 (dd, *J* = 12.2, 6.2 Hz, 2H), 2.66 (s, 3H), 2.39 (s, 3H), 1.93–1.84 (m, 2H), 1.45 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 195.4, 161.7, 156.1, 137.5, 135.8, 134.5, 133.0, 129.5, 128.5, 125.9, 124.5, 123.4, 120.0, 116.8, 112.2, 109.6, 79.1, 47.1, 40.2, 37.3, 29.7, 28.8, 28.4, 26.2, 21.3; HRMS (FAB+) m/z calcd. for $C_{29}H_{33}N_3O_4$ $[M]^+$ 487.25; found: 487.2471.

***tert*-Butyl (*E*)-(3-(7-acetyl-9-(4-methoxystyryl)-3-oxo-1,3-dihydro-2*H*-pyrrolo[3,4-*β*]indolizin-2-yl)propyl)carbamate (11).** ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.48 (d, *J* = 7.2 Hz, 1H), 8.30 (s, 1H), 7.49 (d, *J* = 8.7 Hz, 2H), 7.25 (dd, *J* = 11.7, 5.0 Hz, 2H), 6.95 (d, *J* = 8.7 Hz, 2H), 6.66 (d, *J* = 16.2 Hz, 1H), 5.36 (br s, 1H), 4.57 (s, 2H), 3.87 (s, 3H), 3.72 (t, *J* = 6.3 Hz, 2H), 3.21 (dd, *J*= 12.6, 6.0 Hz, 2H), 2.67 (s, 3H), 1.93–1.87 (m, 2H), 1.45 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 195.4, 161.7, 159.3, 156.1, 135.7, 132.8, 130.1, 128.4, 128.3, 127.2, 124.5, 123.3, 120.1, 115.8, 114.3, 112.4, 109.5, 79.1, 55.4, 47.1, 40.2, 37.4, 31.9, 29.7, 28.4, 26.2; HRMS (FAB+) m/z calcd. for $C_{29}H_{33}N_3O_5$ $[M]^+$ 503.24; found: 503.2420.

***tert*-Butyl (*E*)-(3-(7-acetyl-9-(4-aminostyryl)-3-oxo-1,3-dihydro-2*H*-pyrrolo[3,4-*β*]indolizin-2-yl)propyl)carbamate (12).** 4-nitrostyrene was used as a substrate of cross-coupling reaction and reduced by tin(II) chloride dihydrate in dimethylformamide and 10 volume% acetic acid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.43 (d, *J* = 6.9 Hz, 1H), 8.24 (s, 1H), 7.35 (d, *J* = 8.1 Hz, 2H), 7.21–7.12 (m, 2H), 6.72 (d, *J* = 7.8 Hz, 2H), 6.58 (d, *J* = 16.2 Hz, 1H), 5.39 (br s, 1H), 4.51 (s, 2H), 3.70 (t, *J* = 5.9 Hz, 2H), 3.20 (d, *J* = 5.1 Hz, 2H), 2.65 (s, 3H), 1.90–1.86 (m, 2H), 1.46 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 195.4, 161.7, 156.1, 146.2, 135.4, 132.6, 128.9, 128.1, 127.9, 127.3, 124.4, 123.2, 120.2, 115.3, 114.2, 112.7, 109.4, 79.1, 47.1, 40.1, 37.3, 29.7, 28.8, 28.4, 26.2; HRMS (FAB+) m/z calcd. for $C_{28}H_{32}N_4O_4$ $[M]^+$ 488.24; found: 488.2424.

Methyl (*E*)-9-(4-bromostyryl)-2-(3-((*tert*-butoxycarbonyl)amino) propyl)-3-oxo-2,3-dihydro-1*H*-pyrrolo[3,4-*β*]indolizine-7-carboxylate (13). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.49 (d, *J* = 7.5 Hz, 1H), 8.43 (s, 1H), 7.50 (d, *J* = 8.5 Hz, 2H), 7.39 (d, *J* = 8.5 Hz, 2H), 7.35 (d, *J* = 16.5 Hz, 1H), 7.27 (d, *J* = 8.5 Hz, 1H), 6.58 (d, *J* = 16.0 Hz, 1H), 5.34 (br s, 1H), 4.55 (s, 2H), 3.98 (s, 3H), 3.71 (t, *J* = 6.5 Hz, 2H), 3.19 (dd, *J* = 11.3, 5.8 Hz, 2H), 1.91–1.86 (m, 2H), 1.44 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.7, 161.7, 156.2, 136.4, 133.3, 131.9, 127.4, 126.8, 124.6, 123.3, 122.1, 121.0, 118.7, 111.0, 79.3, 52.6, 47.2, 40.3, 37.4, 29.8, 28.8, 28.5; HRMS (FAB+) m/z calcd. for $C_{28}H_{30}BrN_3O_5$ $[M]^+$ 567.14; found: 567.1369.

Methyl (*E*)-2-(3-((*tert*-butoxycarbonyl)amino)propyl)-3-oxo-9-styryl-2,3-dihydro-1*H*-pyrrolo[3,4-*β*]indolizine-7-carboxylate (14). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.42 (d, *J* = 7.2 Hz, 1H), 8.37 (s, 1H), 7.51 (d, *J* = 7.2 Hz, 2H), 7.41–7.34 (m, 3H), 7.28 (d, *J* = 7.2 Hz, 1H), 7.22 (d, *J* = 7.2 Hz, 1H), 6.61 (d, *J* = 16.2 Hz, 1H), 5.41 (br s, 1H), 4.50 (s, 2H), 3.96 (s, 3H), 3.69 (t, *J* = 6.5 Hz, 2H), 3.20 (br s, 2H), 1.90-1.86 (m, 2H), 1.45 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.6, 161.7, 156.2, 140.8, 137.3, 136.2, 133.1, 128.8, 128.5, 128.2, 127.7, 127.4, 125.9, 124.3, 123.0, 121.7, 120.9, 117.8, 79.2, 52.4, 47.1, 40.2, 37.4, 31.1, 28.8, 28.4; HRMS (FAB+) m/z calcd. for $C_{28}H_{31}N_3O_5$ $[M]^+$ 489.23; found: 489.2264.

Methyl (*E*)-2-(3-((*tert*-butoxycarbonyl)amino)propyl)-9-(4-methoxystyryl)-3-oxo-2,3-dihydro-1*H*-pyrrolo[3,4-*β*]indolizine-7-carboxylate (15). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.40 (d, *J* = 6.0 Hz, 1H), 8.35 (s, 1H), 7.44 (d, *J* = 7.2 Hz, 2H), 7.20–7.13 (m, 2H), 6.92 (d, *J* = 8.4 Hz, 2H), 6.55 (d, *J* = 16.5 Hz, 1H), 5.42 (br s, 1H), 4.48 (s, 2H), 3.95 (s, 3H), 3.85 (s, 3H), 3.68 (t, *J* = 5.0 Hz, 2H), 3.19 (br s, 2H), 1.89-1.85 (m, 2H), 1.44 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.7, 161.8, 159.2, 156.3, 135.9, 132.8, 130.2, 127.9, 127.2, 124.3, 122.8, 121.4, 121.0, 115.8, 114.2, 111.6, 110.6, 79.3, 55.3, 52.4, 47.1, 40.2, 37.4, 31.1, 28.8, 28.4; HRMS (FAB+) m/z calcd. for $C_{29}H_{33}N_3O_6$ $[M]^+$ 519.24; found: 519.2369.

***tert*-Butyl (*E*)-(3-(9-(4-bromostyryl)-7-methyl-3-oxo-1,3-dihydro-2*H*-pyrrolo[3,4-*β*]indolizin-2-yl)propyl)carbamate (16).** ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.42 (d, *J* = 6.9 Hz, 1H), 7.48 (d, *J* = 8.4 Hz, 2H), 7.43–7.32 (m, 3H), 7.27 (s, 1H), 6.62 (d, *J* = 6.9 Hz, 1H), 6.47 (d, *J* = 15.9 Hz, 1H), 5.42 (br s, 1H), 4.52 (s, 2H), 3.70 (t, *J* = 6.3 Hz, 2H), 3.20 (t, *J* = 6.0 Hz, 2H), 2.42 (s, 3H), 1.90–1.86 (m, 2H), 1.45 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.4, 138.7, 137.0, 133.9, 132.8, 131.7, 127.0, 124.9, 124.0, 120.2, 120.1, 119.5, 115.8, 114.9, 105.7, 79.4, 42.3, 40.2, 29.7, 28.9, 28.4, 21.6; HRMS (FAB+) m/z calcd. for $C_{27}H_{30}BrN_3O_3$ $[M]^+$ 523.15; found: 523.1471.

***tert*-Butyl (*E*)-(3-(7-methyl-3-oxo-9-styryl-1,3-dihydro-2*H*-pyrrolo[3,4-*β*]indolizin-2-yl)propyl)carbamate (17).** Copper(II) acetate (2 equiv.) was used as oxidant instead of silver acetate. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.42 (d, *J* = 6.9 Hz, 1H), 7.51 (d, *J* = 7.5 Hz, 2H), 7.44–7.34 (m, 4H), 7.25 (d, *J* = 7.2 Hz, 1H), 6.61-6.53 (m, 2H), 5.48 (br s, 1H), 4.53 (s, 2H), 3.70 (t, *J* = 6.5 Hz, 2H), 3.20 (t, *J*= 5.4 Hz, 2H), 2.42 (s, 3H), 1.90-1.86 (m, 2H), 1.46 (s, 9H); HRMS (FAB+) m/z calcd. for $C_{27}H_{31}N_3O_3$ $[M]^+$ 445.24; found: 445.2365.

***tert*-Butyl (*E*)-(3-(9-(4-methoxystyryl)-7-methyl-3-oxo-1,3-dihydro-2*H*-pyrrolo[3,4-*β*]indolizin-2-yl)propyl)carbamate (18).** ^{1}H NMR (300 MHz, CD_3OD) δ 8.36 (d, *J* = 6.9 Hz, 1H), 7.56 (s, 1H), 7.48 (d, *J* = 8.7 Hz, 2H), 7.25 (d, *J* = 16.2 Hz, 1H), 6.91 (d, *J* = 8.7 Hz, 2H), 6.66 (d, *J* = 6.9 Hz, 1H), 6.58 (d, *J* = 16.2 Hz, 1H), 5.35 (br s, 1H), 4.58 (s, 2H), 3.82 (s, 3H), 3.65 (t, *J* = 8.4 Hz, 2H), 3.14 (t, *J* = 6.8 Hz, 2H), 2.40 (s, 3H), 1.91-1.87 (m, 2H), 1.43 (s, 9H); HRMS (FAB+) m/z calcd. for $C_{28}H_{33}N_3O_4$ $[M]^+$ 475.25; found: 475.2471.

Preparation of *tert*-butyl (3-(7-acetyl-3-oxo-9-(perfluorophenyl)-1,3-dihydro-2*H*-pyrrolo[3,4-*β*]indolizin-2-yl)propyl)carbamate. *tert*-Butyl (3-(7-acetyl-3-oxo-9-(perfluorophenyl)-1,3-dihydro-2*H*-pyrrolo[3,4-*b*]indolizin-2-yl)propyl)carbamate was synthesized by previously reported method.[16]

***tert*-Butyl (3-(7-acetyl-3-oxo-9-(perfluorophenyl)-1,3-dihydro-2*H*-pyrrolo[3,4-*β*]indolizin-2-yl)propyl)carbamate.** ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.68 (d, *J* = 7.2 Hz, 1H), 7.99 (s, 1H), 7.43 (d, *J* = 7.2 Hz, 1H), 5.30 (br s, 1H), 4.40 (s, 2H), 3.70 (t, *J* = 6.3 Hz, 2H), 3.20 (dd, *J*= 12.3, 6.3 Hz, 2H), 2.65 (s, 3H), 1.88–1.83 (m, 2H), 1.45 (s, 9H); HRMS (FAB+) m/z calcd. for $C_{26}H_{24}F_5N_3O_4$ $[M+H]^+$ 538.18; found: 538.1765.

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† Electronic Supplementary Information (ESI) available: Additional figures, full characterization and spectroscopic data of all new compounds, and detailed experimental protocols. See DOI: 10.1039/ c000000x/

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