Rational Improvement of Molar Absorptivity Guided by Oscillator Strength: A Case Study with Furoindolizine-Based Core Skeleton

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1. General Information

a. Basic characterization

$^1$H and $^{13}$C NMR spectra were recorded on an Agilent 400-MR (Agilent Technologies) and Varian Inova-500 (Varian Associates), and 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), br d (broad doublet) etc. Coupling constants were reported in Hz. Low resolution mass spectrometry (LRMS) was obtained by LC/MS system, Finnigan MSQplus Surveyer (Thermo Scientific) or 6120 Quadrupole LC/MS (Agilent Technologies). High resolution mass spectrometry (HRMS) of furoindolizine fluorescence compounds was further confirmed by Ultra High Resolution ESI Q-TOF mass spectrometer (Bruker).

b. Absorption and fluorescence related properties

Absorption spectra and molar absorption coefficient at the absorption maxima of furoindolizine fluorescence compounds were measured by UV-VIS spectrophotometer UV-1650PC (Shimatzu, Japan). Emission spectra was measured by Cary Eclipse Fluorescence spectrophotometer (Varian Associates) and absolute quantum yield was measured by QE-2000 (Otsuka Electronics).

c. Chemical and bio reagents

3-Bromopropylamine hydrobromide, di-tert-butyl dicarbonate, propargyl amine, triethylamine (TEA), bromoacetyl bromide, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), copper(I) iodide (CuI), furo[3,2-b]pyridine, 2-phenylfuro[3,2-b]pyridine, iodobenzene, 4-iodobenzonitrile, 4-idoanisole, 4-bromophenyl methyl sulfone, palladium acetate (PdOAc), bis(triphenylphosphine)palladium(II) dichloride (PdCl$_2$(PPh$_3$)$_2$), silver acetate (AgOAc), potassium acetate (KOAc), tetrabutylammonium fluoride (TBAF) solution, silver trifluoromethanesulfonate, and all terminal alkyne derivatives were purchased from Sigma-Aldrich, Tokyo Chemical Industry Co., Ltd or Acros, and used without further purification. The progress of reaction was monitored using thin-layer chromatography (TLC) (silica gel 60, F$_{254}$ 0.25 mm), and components were visualized by observation under UV light (254 and 365 nm) or by treating the TLC plates with anisaldehyde, KMnO$_4$, and ninhydrin followed by heating. Solvents were purchased from commercial venders and used without further purification. Cell culture reagents including fatal bovine serum, culture media, and antibiotic-antimycotic solution were purchased from GIBCO. MitoTracker Red CMXRos was purchased from Molecular Probes. The culture dish and glass-bottom dish were purchased from CORNING.
d. Quantum mechanical calculations

All quantum mechanical calculations were performed in Gaussian09W. The ground state structures of furoindolizine compounds, Seoul-Fluor (SF) derivatives, and benzocoumarin derivatives were optimized using density functional theory (DFT) at the B3LYP/6-31G* level. The oscillator strength values were calculated through time dependent density functional theory (TD-DFT) with the optimized structures of the ground state. The Energy gap between the S0–S1 states of furoindolizine analogues were calculated based on the optimized structure of the first-excited state to compare with experimental emission properties. Calculation for BODIPY derivatives were performed in the Materials Studio® 4.2 program (Accelrys Software, Inc.) at GAA/ PBE/DNP level in DMol3 to reduce the calculation cost.

e. Fluorescence microscopy

Fluorescence microscopy studies were carried with DeltaVision Elite imaging system (GE Healthcare) equipped with a sCMOS camera. Objective lenses are supported by Olympus IX-71 (Olympus) inverted microscope equipped with Plan APO 60X/Oil (PLAPON60×O), 1.42 NA, WD 0.15 mm. DeltaVision Elite uses a solid state illumination system, InSightSSI fluorescence illumination module. Four-color standard filter set (GE Healthcare, 52-852113-003) was used to detect fluorescence signals.

2. Supporting Figures

![Figure S1](image)

|  | 403 nm | 13,000 | 398 nm | 14,000 | 424 nm | 14,000 |
|---|---|---|---|---|---|---|
| γ(a) | | | | | | |
| 393 nm | f=0.2552 | 392 nm | f=0.2596 | 437 nm | f=0.3098 |
| 346 nm | f=0.0003 | 347 nm | f=0.0570 | 350 nm | f=0.0005 |
| 317 nm | f=0.0007 | 345 nm | f=0.0043 | 339 nm | f=0.1994 |

**Figure S1.** Previous attempts for extension of the π-conjugated system via incorporating naphthyl or styryl groups at the R1 position of indolizine-based SF. (a) Experimental data of the largest absorption maxima and molar absorption coefficients measured in CH2Cl2. (b) Calculated oscillator strength values for S0→S1, S0→S2 and S0→S3, respectively (From top to bottom).
Table S1. Calculated oscillator strength values of the six isomers regarding the $S_0 \rightarrow S_1$ transition.

| Ring | Oxygen | $f_{S_0 \rightarrow S_1}$ |
|------|--------|--------------------------|
| A    | 5      | 0.2739                   |
|      | 6      | 0.2348                   |
| B    | 6      | 0.1317                   |
|      | 7      | 0.0233                   |
| C    | 7      | 0.0622                   |
|      | 8      | 0.0501                   |

Figure S2. Design principles to control the emission wavelength in furoindolizine system.
Table S2. Optimization table for the palladium-mediated cross-coupling reaction on the furoindolizinine-based core skeleton. The reaction yield was evaluated based on the peak integration of $^1$H NMR spectra. $p$-Dimethoxybenzene was used as a reference reagent for $^1$H NMR integration.
Figure S3. Chemical structures and electron density distribution of the HOMO and the LUMO for (a) electron-donating diethylaminophenyl group at the $R^1$ position (12) and (b) electron-donating dimethylaminophenyl group at the $R^2$ position (15), calculated through DFT at the B3LYP/6-31G* level.

Figure S4. A scatter plot of the calculated oscillator strength values ($f$) and the observed molar absorption coefficients ($\varepsilon$).
Figure S5. The calculated oscillator strength values for $S_0 \rightarrow S_1$, $S_0 \rightarrow S_2$ and $S_0 \rightarrow S_3$ (From top to bottom) using DFT/TD-DFT at the B3LYP/6-31G* level and the reported molar absorption coefficients for benzocoumarin derivatives.\cite{1}

| $f$       | $\varepsilon$ | $\chi$ | $\delta$ |
|-----------|---------------|--------|-----------|
| 369 nm f=0.2532 | 382 nm 15,100 | 425 nm 0.0000 | 401 nm 0.0202 |
| 321 nm f=0.0548 | 337 nm 22,500 | 380 nm 0.1505 | 377 nm 0.0000 |
| 387 nm f=0.0000 | 334 nm 0.4606 | 380 nm 0.1005 | 318 nm 0.1583 |

Figure S6. The relationship between the reported molar absorption coefficients and the calculated oscillator strength values for representative BODIPY derivatives. (a) Chemical structures of representative BODIPY derivatives. (b) The reported molar absorption coefficients\cite{2} and the calculated oscillator strength values for the $S_0 \rightarrow S_1$ transition. (c) A scatter plot of calculated oscillator strength values and reported molar absorption coefficients.
3. Synthetic Procedure and Compound Characterization

Preparation of tert-butyl (3-(prop-2-yn-1-ylamino)propyl)carbamate was conducted through the previous synthetic report. [3]

Preparation of tert-butyl (3-(2-bromo-N-(prop-2-yn-1-yl)acetamido)propyl)carbamate

To a stirred solution of bromoacetyl bromide (2.0 equiv.) in anhydrous CH₂Cl₂ (0.1 M) at −78 °C under argon, added dropwise a tert-butyl (3-(prop-2-yn-1-ylamino)propyl)carbamate (1 equiv.) and 3 equiv. of triethylamine (TEA) in CH₂Cl₂ (0.1 M) over a period of 1 h. The solution was stirred at −78 °C for 2 h. When the reaction was completed checked by TLC, saturated NaHCO₃(aq) was added to the solution and the organic material was extracted with CH₂Cl₂. The combined organic extracts were dried over Na₂SO₄(s), and concentrated in vacuo after filtration. The residue was purified by silica-gel flash column chromatography to afford the desired product. (Transparent oil, Y: 83%)

tert-Butyl (3-(2-bromo-N-(prop-2-yn-1-yl)acetamido)propyl)carbamate

\[1\] NMR (2:1 rotamer ratio, asterisks denote minor rotamer peaks, 400 MHz, CDCl₃) \(\delta\) 5.23 (br s, 1H), 4.76* (br s, 1H), 4.23* (d, \(J = 2.0\) Hz, 2H), 4.14 (d, \(J = 2.0\) Hz, 2H), 3.96 (s, 2H), 3.89* (s, 2H), 3.56–3.52 (m, 2H), 3.56–3.52* (m, 2H), 3.23–3.18* (m, 2H), 3.13–3.09 (m, 2H), 2.38 (br s, 1H), 2.26* (br s, 1H), 1.94–1.88* (m, 2H), 1.79–1.73 (m, 2H), 1.45* (s, 9H), 1.44 (s, 9H); \(^{13}\)C NMR (100 MHz, CDCl₃) \(\delta\) 167.5, 166.4*, 156.2, 79.1, 78.2*, 78.0, 73.7, 72.6*, 45.9*, 44.2, 38.2, 38.0*, 37.1, 34.9*, 29.3*, 28.5, 28.5*, 27.6, 26.1, 25.9*; LRMS (ESI) \(m/z\) caled for C₁₅H₂₂BrN₂O₃ [M+H]⁺: 333.08; Found: 332.92.
General procedure for preparation of furopyridine derivatives

a) Terminal alkyne derivatives required to prepare various furopyridine derivatives were commercially available, except 1-(4-ethynylphenyl)ethanone and 4-(4-ethylphenyl)morpholine, which were synthesized by reported procedure.\[4,5\]

b) Preparation of 2-bromopyridin-3-yl acetate was conducted refer to the reported synthetic procedure.\[6\]

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\begin{center}
\begin{tikzpicture}
  \node[anchor=center] (image) at (0,0) {
    \includegraphics[width=\textwidth]{./image.png}
  };
\end{tikzpicture}
\end{center}
```

To a suspension of 2-bromopyridin-3-yl acetate (1.0 equiv.), PdCl$_2$(PPh$_3$)$_2$ (3 mol%), and CuI (6 mol %) in THF (0.1 M), in the presence of TEA (15.0 equiv.), was added a terminal alkyne derivative (2.0 equiv.) under argon atmosphere with vigorous stirring at 50°C. After the reaction was completed checked by TLC, the resulting solution was filtered through Celite and concentrated in vacuo. After that, to the solution of filtered residue in MeOH (0.1 M), were added potassium carbonate (3.0 equiv.) and silver triflate (10 mol%) with stirring at room temperature. When the reaction was completed checked by TLC, brine was added to the solution and the organic material was extracted with ethyl acetate. The combined organic extracts were dried over Na$_2$SO$_4$(s), and concentrated under reduced pressure after filtration. The residue was purified by silica-gel flash column chromatography to afford the desired furopyridine derivatives.

4-(Furo[3,2-b]pyridin-2-yl)benzonitrile

Yield: 45%; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.59 (d, $J = 4.4$ Hz, 1H), 8.00 (d, $J = 8.4$ Hz, 2H), 7.81 (d, $J = 8.4$ Hz, 1H), 7.77 (d, $J = 8.8$ Hz, 2H), 7.37 (s, 1H), 7.28 (dd, $J = 4.4$, 8.4 Hz, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 157.1, 148.6, 148.4, 146.9, 133.8, 132.8, 125.7, 120.0, 118.6, 118.4, 112.7, 105.4; LRMS (ESI) m/z calcd for C$_{14}$H$_9$N$_2$O [M+H]$^+$: 221.07; Found: 220.88.
4-(Furo[3,2-b]pyridin-2-yl)-N,N-dimethylaniline

Yield: 77%; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.46 (br s, 1H), 7.75 (d, $J = 8.8$ Hz, 2H), 7.66 (d, $J = 8.0$ Hz, 1H), 7.09 (dd, $J = 4.6$, 8.2 Hz, 1H), 6.97 (s, 1H), 6.74 (d, $J = 8.8$ Hz, 2H), 3.00 (s, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 161.0, 151.2, 150.0, 147.7, 145.6, 126.7, 117.7, 117.5, 117.0, 112.0, 99.0, 40.3; LRMS (ESI) m/z calcd for C$_{15}$H$_{15}$N$_2$O $[M+H]^+$: 239.12; Found: 238.98.

2-(4-Morpholinophenyl)furo[3,2-b]pyridine

Yield: 63%; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.48 (d, $J = 4.0$ Hz, 1H), 7.81 (d, $J = 9.2$ Hz, 2H), 7.71 (d, $J = 8.0$ Hz, 1H), 7.15 (dd, $J = 4.6$, 8.2 Hz, 1H), 7.05 (s, 1H), 6.98 (d, $J = 8.8$ Hz, 2H), 3.89 (t, $J = 4.8$ Hz, 4H), 3.27 (t, $J = 5.0$ Hz, 4H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 160.2, 152.1, 149.7, 147.9, 145.9, 126.7, 121.0, 118.2, 117.4, 115.1, 100.4, 66.8, 48.5; LRMS (ESI) m/z calcd for C$_{17}$H$_{17}$N$_2$O$_2$ $[M+H]^+$: 281.13; Found: 281.03.

1-(4-(Furo[3,2-b]pyridin-2-yl)phenyl)ethanone

Yield: 54%; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.57 (br s, 1H), 8.06 (d, $J = 8.0$ Hz, 2H), 7.98 (d, $J = 8.0$ Hz, 2H), 7.80 (d, $J = 8.4$ Hz, 1H), 7.35 (s, 1H), 7.28–7.24 (m, 1H), 2.65 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 197.3, 158.2, 148.6, 148.5, 146.5, 137.4, 133.8, 129.1, 125.4, 119.6, 118.3, 104.6, 26.8; LRMS (ESI) m/z calcd for C$_{15}$H$_{12}$NO$_2$ $[M+H]^+$: 238.09; Found: 238.1.

2-(Triisopropylsilyl)furo[3,2-b]pyridine

Yield: 90%; $^1$H NMR (500 MHz, acetone-$d_6$) $\delta$ 8.52 (dd, $J = 1.3$, 4.8 Hz, 1H), 7.91 (d, $J = 8.5$ Hz, 1H), 7.32 (d, $J = 1.0$ Hz, 1H), 7.28 (dd, $J = 4.5$, 8.5 Hz, 1H), 1.50–1.44 (m, 3H), 1.22–1.13 (m, 18H); $^{13}$C NMR (125 MHz, acetone-$d_6$) $\delta$ 166.0, 151.3, 149.0, 146.7, 120.4, 119.8, 118.4, 18.9, 11.7; LRMS (ESI) m/z calcd for C$_{16}$H$_{26}$NOSi $[M+H]^+$: 276.18; Found: 276.2.
General procedure for preparation of furoindolizine-based core skeletons

![Diagram]

To a solution of tert-butyl (3-(2-bromo-N-(prop-2-yn-1-yl)acetamido)propyl)carbamate (1.2 equiv.) in acetonitrile (0.1M), was added a furopyridine derivative (1.0 equiv.), and the solution was stirred at 80°C overnight. After the complete consumption of starting materials, copper iodide (1.0 equiv.) was added to a reaction mixture followed by slow addition of 1,8-diazabicycloundec-7-ene (DBU) (3.0 equiv.) at room temperature with vigorous stirring. When the reaction was completed checked by TLC, the resulting mixture was filtered through the short bed of silica gel and concentrated in vacuo. The residue was purified by silica-gel flash column chromatography to afford the desired furoindolizine-based core skeletons. Some furopyridine derivatives used different metal sources instead of copper iodide; AgOTf was used in the case of R2 = 4-(acetyl)Ph group, and Ag2O was used in the case of R2 = 4-(NMe2)Ph and 4-(morpholino)Ph.

**tert-Butyl (3-(9-oxo-7H-furo[3,2-e]pyrrolo[3,4-b]indolizin-8(9H)-yl)propyl)carbamate (01)**

Yield: 19%; 1H NMR (400 MHz, CDCl3) δ 7.85 (d, J = 2.0 Hz, 1H), 7.62 (d, J = 2.4 Hz, 1H), 7.29–7.23 (m, 2H), 6.45 (s, 1H), 5.44 (br s, 1H), 4.35 (s, 2H), 3.66 (t, J = 6.2 Hz, 2H), 3.17 (q, J = 6.1 Hz, 2H), 1.86–1.79 (m, 2H), 1.44 (s, 9H); 13C NMR (100 MHz, CDCl3) δ 162.2, 156.2, 143.3, 143.1, 138.4, 135.8, 125.7, 121.6, 114.3, 109.7, 104.8, 94.9, 79.0, 46.6, 40.1, 37.4, 28.9, 28.5; HRMS (ESI) m/z calcd for C20H23N3NaO4 [M+Na]+: 392.1581; Found: 392.1581.
**tert-Butyl (3-(2-(4-cyanophenyl)-9-oxo-7H-furo[3,2-e]pyrrolo[3,4-b]indolizin-8(9H)-yl)propyl)carbamate (05)**

Yield: 49%; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.19 (s, 1H), 7.92 (d, $J = 8.8$ Hz, 2H), 7.68 (d, $J = 8.8$ Hz, 2H), 7.35 (d, $J = 9.6$ Hz, 1H), 7.26 (d, $J = 8.8$ Hz, 1H), 6.51 (s, 1H), 5.36 (br s, 1H), 4.40 (s, 2H), 3.68 (t, $J = 6.4$ Hz, 2H), 3.21 (br d, $J = 5.6$ Hz, 2H), 1.87–1.84 (m, 2H), 1.46 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 162.1, 156.2, 151.8, 143.9, 138.3, 136.1, 133.9, 132.7, 127.1, 124.7, 122.3, 118.9, 115.9, 111.3, 109.2, 102.1, 95.9, 79.2, 46.8, 40.3, 37.6, 29.1, 28.6; HRMS (ESI) $m/z$ calcd for C$_{27}$H$_{26}$N$_4$NaO$_4$ [M+Na]$^+$: 493.1846; Found: 493.1847.

**tert-Butyl (3-(9-oxo-2-phenyl-7H-furo[3,2-e]pyrrolo[3,4-b]indolizin-8(9H)-yl)propyl)carbamate (09)**

Yield: 35%; $^1$H NMR (400 MHz, CD$_2$Cl$_2$) $\delta$ 8.08 (s, 1H), 7.92 (d, $J = 7.2$ Hz, 2H), 7.47 (t, $J = 7.6$ Hz, 2H), 7.38–7.31 (m, 3H), 6.51 (s, 1H), 5.50 (br s, 1H), 4.37 (s, 2H), 3.65 (t, $J = 6.2$ Hz, 2H), 3.13 (q, $J = 6.3$ Hz, 2H), 1.83–1.76 (m, 2H), 1.44 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 162.3, 156.3, 154.4, 142.9, 138.6, 136.0, 128.9, 128.6, 127.5, 124.7, 121.6, 114.1, 109.4, 99.4, 95.1, 79.1, 46.7, 40.3, 37.6, 29.0, 28.6; HRMS (ESI) $m/z$ calcd for C$_{26}$H$_{27}$N$_3$NaO$_4$ [M+Na]$^+$: 468.1894; Found: 468.1893.

**tert-Butyl (3-(2-(4-(dimethylamino)phenyl)-9-oxo-7H-furo[3,2-e]pyrrolo[3,4-b]indolizin-8(9H)-yl)propyl)carbamate (13)**

Yield: 26%; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.87 (s, 1H), 7.78 (d, $J = 9.2$ Hz, 2H), 7.25 (d, $J = 6.4$ Hz, 1H), 7.19 (d, $J = 9.6$ Hz, 1H), 6.77 (d, $J = 8.8$ Hz, 2H), 6.42 (s, 1H), 5.40 (br s, 1H), 4.36 (s, 2H), 3.67 (t, $J = 6.2$ Hz, 2H), 3.19 (br d, $J = 5.6$ Hz, 2H), 3.03 (s, 6H), 1.85–1.82 (m, 2H), 1.45 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 162.4, 156.3, 155.8, 150.7, 142.0, 138.9, 136.1, 128.1, 126.1, 121.1, 118.3, 112.5, 112.2, 109.4, 96.4, 94.5, 79.1, 46.7, 40.4, 40.3, 37.5, 29.1, 28.6; HRMS (ESI) $m/z$ calcd for C$_{28}$H$_{32}$N$_4$NaO$_4$ [M+Na]$^+$: 511.2316; Found: 511.2317.
**tert-Butyl (3-(2-(4-morpholinophenyl)-9-oxo-7H-furo[3,2-e]pyrrolo[3,4-b]indolizin-8(9H)-yl)propyl)carbamate (17)**

Yield: 22%; ¹H NMR (400 MHz, CDCl₃) δ 7.94 (s, 1H), 7.81 (d, J = 8.4 Hz, 2H), 7.28–7.21 (m, 2H), 6.96 (d, J = 8.4 Hz, 2H), 6.44 (s, 1H), 5.38 (br s, 1H), 4.37 (s, 2H), 3.89 (t, J = 4.6 Hz, 4H), 3.67 (t, J = 6.4 Hz, 2H), 3.25 (t, J = 4.6 Hz, 4H), 3.20 (q, J = 5.9 Hz, 2H), 1.87–1.81 (m, 2H), 1.45 (s, 9H); ¹³C NMR (100 MHz, CDCl₃) δ 162.4, 156.3, 155.0, 151.4, 142.4, 138.8, 136.1, 127.9, 126.0, 121.6, 121.3, 115.3, 113.2, 109.4, 97.5, 94.7, 79.1, 66.9, 48.7, 46.7, 40.3, 37.6, 29.1, 28.6; HRMS (ESI) m/z calcd for C₃₀H₃₄N₄NaO₅ [M+Na]⁺: 553.2421; Found: 553.2421.

**tert-Butyl (3-(2-(4-acetylphenyl)-9-oxo-7H-furo[3,2-e]pyrrolo[3,4-b]indolizin-8(9H)-yl)propyl)carbamate**

Yield: 29%; ¹H NMR (400 MHz, CDCl₃) δ 8.07 (s, 1H), 7.92 (d, J = 8.4 Hz, 2H), 7.84 (d, J = 8.0 Hz, 2H), 7.26 (d, J = 9.6 Hz, 1H), 7.20 (d, J = 9.2 Hz, 1H), 6.42 (s, 1H), 5.41 (br s, 1H), 4.33 (s, 2H), 3.66 (t, J = 6.2 Hz, 2H), 3.21 (br d, J = 6.0 Hz, 2H), 2.59 (s, 3H), 1.87–1.84 (m, 2H), 1.46 (s, 9H); ¹³C NMR (100 MHz, CDCl₃) δ 197.2, 162.0, 156.2, 152.7, 143.5, 138.3, 136.1, 135.9, 133.9, 128.9, 127.1, 124.2, 121.9, 115.2, 109.1, 101.3, 95.6, 79.2, 46.7, 40.3, 37.6, 29.0, 28.5, 26.6; LRMS (ESI) m/z calcd for C₂₈H₃₀N₃O₅ [M+H]⁺: 488.2; Found: 488.2.

**tert-Butyl (3-(9-oxo-2-(triisopropylsilyl)-7H-furo[3,2-e]pyrrolo[3,4-b]indolizin-8(9H)-yl)propyl)carbamate**

Yield: 70%; ¹H NMR (400 MHz, CDCl₃) δ 8.07 (s, 1H), 7.29–7.23 (m, 2H), 6.44 (s, 1H), 5.31 (br s, 1H), 4.35 (s, 2H), 3.67 (t, J = 6.4 Hz, 2H), 3.19 (q, J = 6.1 Hz, 2H), 1.88–1.83 (m, 2H), 1.48–1.40 (m, 12H), 1.17–1.15 (m, 18H); ¹³C NMR (100 MHz, CDCl₃) δ 162.3, 160.0, 156.3, 146.9, 138.6, 135.7, 126.4, 121.8, 115.2, 114.1, 110.0, 94.8, 79.1, 46.6, 40.3, 37.7, 29.0, 28.5, 18.7, 11.2; LRMS (ESI) m/z calcd for C₂₉H₄₄N₃O₄Si [M+H]⁺: 526.31; Found: 526.3.
Procedure for preparation of R¹ group embedded furoindolizine compounds (Pd coupling reaction)

Preparation of N,N-diethyl-4-iodoaniline was conducted through the reported procedure.[7]

For general iodo-aryl moieties

Condition A. To a solution of a furoindolizine-based core skeleton in dimethylformamide (0.1 M), were added a iodo-aryl derivative (2.0 equiv.), bis(triphenylphosphine)palladium(II) dichloride (PdCl₂(PPh₃)₂, 10 mol%), silver acetate (1.5 equiv.), and potassium acetate (3.0 equiv.), and the solution was stirred at 100 °C overnight. When the reaction was completed checked by TLC, the reaction mixture was filtered through the short bed of silica gel and concentrated in vacuo. The residue was purified by silica-gel flash column chromatography to afford the desired products. (** at isolated yield indicates 10 mol % of PdCl₂(PPh₃)₂ was applied additionally to completely convert the starting materials)

For N,N-diethyl-4-iodoaniline and 1-iodo-4-methoxybenzene

Condition B. To a solution of a furoindolizine-based core skeleton in dimethylformamide (0.1 M), were added a iodo-aryl derivative (2.0 equiv.), palladium acetate (20 mol%), and potassium acetate (3.0 equiv.), and the solution was stirred at 80 °C overnight. When the reaction was completed checked by TLC, the reaction mixture was filtered through the short bed of silica gel and concentrated in vacuo. The residue was purified by silica-gel flash column chromatography to afford the desired products.
**tert-Butyl (3-(2,6-bis(4-cyanophenyl)-9-oxo-7H-furo[3,2-e]pyrrolo[3,4-b]indolin-8(9H)-yl)propyl)carbamate (06)**

Yield: 80%**; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.29 (s, 1H), 7.96 (d, 8.8 Hz, 2H), 7.76–7.65 (m, 7H), 7.43 (d, \(J = 9.6\) Hz, 1H), 5.24 (br s, 1H), 4.57 (s, 2H), 3.73 (t, \(J = 6.6\) Hz, 2H), 3.23–3.20 (m, 2H), 1.91–1.88 (m, 2H), 1.45 (s, 9H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 161.7, 156.2, 152.8, 144.2, 139.3, 135.1, 134.9, 133.6, 133.1, 132.8, 127.7, 127.6, 125.0, 122.8, 119.1, 118.7, 114.3, 111.9, 111.0, 109.6, 109.4, 102.1, 79.4, 47.0, 40.6, 37.7, 29.1, 28.6; HRMS (ESI) \(m/z\) calcd for C\(_{34}H_{29}N_5NaO_4\) [M+Na]\(^+\): 594.2112; Found: 594.2111.

**tert-Butyl (3-(2-(4-cyanophenyl)-9-oxo-6-phenyl-7H-furo[3,2-e]pyrrolo[3,4-b]indolin-8(9H)-yl)propyl)carbamate (07)**

Yield: 61%; \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 8.24 (s, 1H), 7.92 (d, \(J = 8.5\) Hz, 2H), 7.68 (d, \(J = 9.0\) Hz, 3H), 7.56 (d, \(J = 7.0\) Hz, 2H), 7.50–7.47 (m, 2H), 7.34–7.29 (m, 2H), 5.33 (br s, 1H), 4.53 (s, 2H), 3.71 (t, \(J = 6.5\) Hz, 2H), 3.21 (br d, \(J = 6.0\) Hz, 2H), 1.88–1.86 (m, 2H), 1.45 (s, 9H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 162.0, 156.2, 152.1, 144.2, 134.8, 134.4, 134.3, 133.8, 132.7, 129.3, 127.6, 127.3, 126.6, 124.8, 122.0, 118.9, 115.1, 111.5, 111.4, 109.7, 102.2, 79.3, 47.0, 40.5, 37.6, 29.1, 28.6; HRMS (ESI) \(m/z\) calcd for C\(_{33}H_{30}N_4NaO_4\) [M+Na]\(^+\): 569.2159; Found: 569.2160.

**tert-Butyl (3-(2-(4-cyanophenyl)-6-(4-(diethylamino)phenyl)-9-oxo-7H-furo[3,2-e]pyrrolo[3,4-b]indolin-8(9H)-yl)propyl)carbamate (08)**

Yield: 27%; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.20 (s, 1H), 7.91 (d, \(J = 8.4\) Hz, 2H), 7.67 (d, \(J = 8.4\) Hz, 2H), 7.64 (d, \(J = 10.0\) Hz, 1H), 7.41 (d, \(J = 9.2\) Hz, 2H), 7.22 (d, \(J = 10.0\) Hz, 1H), 6.79 (d, \(J = 8.8\) Hz, 2H), 5.37 (br s, 1H), 4.49 (s, 2H), 3.70 (t, \(J = 6.4\) Hz, 2H), 3.42 (q, \(J = 7.0\) Hz, 4H), 3.20 (br d, \(J = 5.6\) Hz, 2H), 1.87–1.84 (m, 2H), 1.45 (s, 9H), 1.22 (t, \(J = 7.0\) Hz, 6H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 162.2, 156.3, 151.7, 146.6, 144.2, 134.4, 134.0, 133.7, 132.7, 128.7, 127.2, 124.7, 121.6, 121.0, 119.0, 115.6, 112.3, 112.2, 111.2, 108.7, 102.3, 79.3, 47.0, 44.6, 40.5, 37.6, 29.1, 28.6, 12.8; HRMS (ESI) \(m/z\) calcd for C\(_{37}H_{40}N_5O_4\) [M+H]\(^+\): 618.3075; Found: 618.3077.
**tert-Butyl (3-(6-(4-cyanophenyl)-9-oxo-2-phenyl-7H-furo[3,2-e]pyrrolo[3,4-b]indolizin-8(9H)-yl)propyl)carbamate (10)**

Yield: 81%; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.06 (s, 1H), 7.83 (d, $J = 8.0$ Hz, 2H), 7.69 (d, $J = 8.0$ Hz, 2H), 7.61–7.55 (m, 3H), 7.44 (t, $J = 7.6$ Hz, 2H), 7.38–7.34 (m, 2H), 5.30 (br s, 1H), 4.49 (s, 2H), 3.70 (t, $J = 6.2$ Hz, 2H), 3.22 (br d, $J = 6.0$ Hz, 2H), 1.90–1.86 (m, 2H), 1.45 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 161.7, 156.2, 155.3, 143.1, 139.6, 135.3, 134.8, 132.9, 129.6, 129.1, 129.0, 128.0, 127.2, 124.8, 122.2, 119.3, 112.5, 111.1, 108.8, 108.6, 99.3, 79.3, 47.0, 40.5, 37.7, 29.1, 28.6; HRMS (ESI) m/z calcd for C$_{33}$H$_{30}$N$_4$NaO$_4$ [M+Na]$^+$: 569.2159; Found: 569.2158.

**tert-Butyl (3-(9-oxo-2,6-diphenyl-7H-furo[3,2-e]pyrrolo[3,4-b]indolizin-8(9H)-yl)propyl)carbamate (11)**

Yield: 67%; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.13 (s, 1H), 7.90 (d, $J = 7.2$ Hz, 2H), 7.61 (d, $J = 9.6$ Hz, 1H), 7.56 (d, $J = 8.0$ Hz, 2H), 7.49–7.43 (m, 4H), 7.37–7.28 (m, 3H), 5.37 (br s, 1H), 4.50 (s, 2H), 3.69 (t, $J = 6.2$ Hz, 2H), 3.21 (br d, $J = 6.4$ Hz, 2H), 1.87–1.83 (m, 2H), 1.45 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 162.2, 156.3, 154.7, 143.1, 135.1, 134.7, 134.4, 130.0, 129.2, 129.0, 128.8, 127.7, 127.5, 126.3, 124.8, 121.4, 113.3, 110.7, 110.0, 99.5, 79.2, 46.9, 40.4, 37.6, 29.0, 28.6; HRMS (ESI) m/z calcd for C$_{32}$H$_{31}$N$_3$NaO$_4$ [M+Na]$^+$: 544.2207; Found: 544.2207.

**tert-Butyl (3-(6-(4-(diethylamino)phenyl)-9-oxo-2-phenyl-7H-furo[3,2-e]pyrrolo[3,4-b]indolizin-8(9H)-yl)propyl)carbamate (12)**

Yield: 30%; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.12 (s, 1H), 7.91 (d, $J = 8.4$ Hz, 2H), 7.59 (d, $J = 10.0$ Hz, 1H), 7.47–7.41 (m, 4H), 7.36–7.32 (m, 1H), 7.27–7.25 (m, 1H), 6.79 (d, $J = 8.4$ Hz, 2H), 5.42 (br s, 1H), 4.48 (s, 2H), 3.69 (t, $J = 6.2$ Hz, 2H), 3.41 (q, $J = 7.0$ Hz, 4H), 3.20 (br d, $J = 5.6$ Hz, 2H), 1.86–1.83 (m, 2H), 1.45 (s, 9H), 1.22 (t, $J = 7.0$ Hz, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 162.4, 156.3, 154.3, 146.4, 143.2, 134.7, 133.7, 130.2, 128.9, 128.7, 128.6, 127.6, 124.7, 121.4, 120.9, 113.8, 112.4, 111.3, 109.1, 99.6, 79.2, 46.9, 44.6, 40.4, 37.6, 29.0, 28.6, 12.8; HRMS (ESI) m/z calcd for C$_{36}$H$_{41}$N$_4$O$_4$ [M+H]$^+$: 593.3122; Found: 593.3121.
**tert-Butyl (3-(6-(4-cyanophenyl)-2-(4-(dimethylamino)phenyl)-9-oxo-7H-furo[3,2-e]pyrrolo[3,4-b]indolizin-8(9H)-yl)propyl)carbamate (14)**

Yield: 86%; 1H NMR (400 MHz, CDCl3) δ 7.92 (s, 1H), 7.77 (d, J = 9.2 Hz, 2H), 7.71 (d, J = 8.4 Hz, 2H), 7.64 (d, J = 8.8 Hz, 2H), 7.55 (d, J = 10.0 Hz, 1H), 7.40 (d, J = 10.0 Hz, 1H), 6.76 (d, J = 9.2 Hz, 2H), 5.29 (br s, 1H), 4.53 (t, J = 8.4 Hz, 2H), 3.71 (q, J = 6.1 Hz, 2H), 3.05 (s, 6H), 1.90–1.86 (m, 2H), 1.44 (s, 9H); 13C NMR (100 MHz, CDCl3) δ 161.9, 157.0, 156.3, 151.0, 142.4, 140.0, 135.8, 135.0, 133.0, 128.9, 127.2, 126.4, 121.8, 119.4, 117.7, 112.2, 111.2, 110.8, 108.6, 108.0, 96.2, 79.3, 47.0, 40.5, 40.4, 37.7, 29.1, 28.6; HRMS (ESI) m/z calcd for C35H35N5NaO4 [M+Na]+: 612.2581; Found: 612.2581.

**tert-Butyl (3-(2-(4-(dimethylamino)phenyl)-9-oxo-6-phenyl-7H-furo[3,2-e]pyrrolo[3,4-b]indolizin-8(9H)-yl)propyl)carbamate (15)**

Yield: 81%**; 1H NMR (400 MHz, CDCl3) δ 7.94 (s, 1H), 7.80 (d, J = 8.8 Hz, 2H), 7.59–7.56 (m, 3H), 7.47 (t, J = 7.8 Hz, 2H), 7.34–7.28 (m, 2H), 6.77 (d, J = 9.2 Hz, 2H), 5.38 (br s, 1H), 4.52 (s, 2H), 3.70 (t, J = 6.2 Hz, 2H), 3.21 (q, J = 6.0 Hz, 2H), 3.04 (s, 6H), 1.88–1.84 (m, 2H), 1.44 (s, 9H); 13C NMR (100 MHz, CDCl3) δ 162.4, 156.3, 156.2, 150.8, 142.4, 140.0, 135.5, 135.0, 134.5, 129.2, 128.5, 127.5, 126.2, 126.1, 120.9, 118.2, 112.2, 111.6, 110.1, 110.0, 96.4, 79.2, 46.9, 40.5, 40.4, 37.6, 29.0, 28.6; HRMS (ESI) m/z calcd for C34H37N4O4 [M+H]+: 565.2809; Found: 565.2807.

**tert-Butyl (3-(2-(4-acetylphenyl)-6-(4-methoxyphenyl)-9-oxo-7H-furo[3,2-e]pyrrolo[3,4-b]indolizin-8(9H)-yl)propyl)carbamate (19)**

Yield: 17%; 1H NMR (500 MHz, CD2Cl2) δ 8.28 (s, 1H), 8.04 (d, J = 8.5 Hz, 2H), 8.01 (d, J = 9.0 Hz, 2H), 7.66 (d, J = 10.0 Hz, 1H), 7.52 (d, J = 9.0 Hz, 2H), 7.35 (d, J = 10.0 Hz, 1H), 7.03 (d, J = 9.0 Hz, 2H), 5.45 (br s, 1H), 4.51 (s, 2H), 3.86 (s, 3H), 3.69 (t, J = 6.5 Hz, 2H), 3.16 (q, J = 6.2 Hz, 2H), 2.62 (s, 3H), 1.85–1.80 (m, 2H), 1.43 (s, 9H); HRMS (ESI) m/z calcd for C35H35N3NaO6 [M+Na]+: 616.2418; Found: 616.2417.
**tert-Butyl (3-(6-(4-cyanophenyl)-9-oxo-2-(triisopropylsilyl)-7H-furo[3,2-e]pyrrolo[3,4-b]indolizin-8(9H)-yl)propyl)carbamate**

Yield: 78%; 1H NMR (400 MHz, CDCl3) δ 8.17 (s, 1H), 7.73 (d, J = 8.8 Hz, 2H), 7.66–7.62 (m, 3H), 7.45 (d, J = 10.0 Hz, 1H), 5.21 (br s, 1H), 4.54 (s, 2H), 3.71 (t, J = 6.6 Hz, 2H), 3.21 (br d, J = 6.4 Hz, 2H), 1.90–1.87 (m, 2H), 1.50–1.42 (m, 12H), 1.17 (d, J = 7.2 Hz, 18H); 13C NMR (100 MHz, CDCl3) δ 161.8, 161.6, 156.2, 147.2, 139.9, 135.3, 134.5, 133.0, 127.4, 127.0, 122.4, 119.3, 115.1, 112.5, 111.8, 108.8, 108.5, 79.2, 46.9, 40.5, 37.8, 29.0, 28.5, 18.7, 11.2; LRMS (ESI) m/z calcd for C38H47N4O4Si [M+H]+: 627.34; Found: 627.3.

**tert-Butyl (3-(9-oxo-6-phenyl-2-(triisopropylsilyl)-7H-furo[3,2-e]pyrrolo[3,4-b]indolizin-8(9H)-yl)propyl)carbamate**

Yield: 84%**; 1H NMR (400 MHz, CDCl3) δ 8.14 (s, 1H), 7.63 (d, J = 10.0 Hz, 1H), 7.57 (d, J = 8.0 Hz, 2H), 7.47 (t, J = 7.8 Hz, 2H), 7.34 (d, J = 9.6 Hz, 1H), 7.29 (t, J = 8.0 Hz, 1H), 5.30 (br s, 1H), 4.51 (s, 2H), 3.70 (t, J = 6.6 Hz, 2H), 3.20 (q, J = 6.0 Hz, 2H), 1.88–1.85 (m, 2H), 1.49–1.42 (m, 12H), 1.17 (d, J = 7.2 Hz, 18H); 13C NMR (100 MHz, CDCl3) δ 162.2, 160.5, 156.3, 147.1, 135.0, 134.9, 134.1, 129.2, 127.5, 126.7, 126.2, 121.5, 115.2, 113.2, 110.6, 110.4, 79.1, 46.8, 40.4, 37.7, 29.0, 28.5, 18.8, 11.2; LRMS (ESI) m/z calcd for C35H48N3O4Si [M+H]+: 602.34; Found: 602.3.

**tert-Butyl (3-(6-(4-(diethylamino)phenyl)-9-oxo-2-(triisopropylsilyl)-7H-furo[3,2-e]pyrrolo[3,4-b]indolizin-8(9H)-yl)propyl)carbamate**

Yield: 32%; 1H NMR (400 MHz, CD2Cl2) δ 8.12 (s, 1H), 7.60 (d, J = 9.6 Hz, 1H), 7.42 (d, J = 8.8 Hz, 2H), 7.29 (d, J = 9.8 Hz, 1H), 6.78 (d, J = 9.2 Hz, 2H), 5.47 (br s, 1H), 4.48 (s, 2H), 3.66 (t, J = 6.6 Hz, 2H), 3.40 (q, J = 7.1 Hz, 4H), 3.14 (q, J = 6.1 Hz, 2H), 1.84–1.78 (m, 2H), 1.49–1.42 (m, 12H), 1.21–1.18 (m, 24H); 13C NMR (100 MHz, CD2Cl2) δ 162.4, 160.1, 156.3, 147.4, 146.7, 134.6, 133.7, 128.7, 126.6, 121.8, 121.5, 115.5, 114.0, 112.6, 111.3, 109.7, 78.9, 47.0, 44.8, 40.5, 37.7, 29.1, 28.5, 18.8, 12.8, 11.5; LRMS (ESI) m/z calcd for C39H57N4O4Si [M+H]+: 673.41; Found: 673.4.
General procedure for TIPS deprotection reaction
(To generate 02, 03, and 04)

To a stirred solution of TIPS protected furoindolizine-based core skeleton in THF (0.1 M) at
–40°C, was slowly added tetrabutylammonium fluoride solution (1.0 M in THF, 1.5 equiv.)
which was cooled down to –10 °C. Stirred reaction mixture was allowed to warm up to 0 °C for
1 h. When the reaction was completed checked by TLC, brine was added to the solution and the
organic material was extracted with ethyl acetate. The combined organic extracts were dried over
Na₂SO₄(s), and concentrated under reduced pressure after filtration. The residue was purified by
silica-gel flash column chromatography to afford the desired product.

**tert-Butyl (3-(6-(4-cyanophenyl)-9-oxo-7H-furo[3,2-e]pyrrolo[3,4-b]indolizin-8(9H)-yl)propyl)carbamate (02)**

Yield: 82%; ¹H NMR (400 MHz, CD₂Cl₂) δ 7.91 (d, J = 2.0 Hz, 1H), 7.74 (d, J = 8.4 Hz, 2H), 7.71 (d, J = 2.0 Hz, 1H), 7.65 (d, J = 8.4 Hz, 3H), 7.41 (d, J = 9.6 Hz, 2H), 5.42 (br s, 1H), 4.51 (s, 2H), 3.66 (t, J = 6.6 Hz, 2H), 3.13 (q, J = 6.5 Hz, 2H), 1.85–1.78 (m, 2H), 1.41 (s, 9H); ¹³C NMR (100 MHz, CD₂Cl₂) δ 161.9, 156.2, 144.6, 143.8, 139.9, 135.4, 135.1, 133.2, 127.6, 126.5, 122.7, 119.5, 113.3, 111.6, 109.1, 108.9, 105.1, 79.1, 47.1, 40.5, 37.6, 29.1, 28.5; HRMS (ESI) m/z calcd for C₂₇H₂₆N₄NaO₄
[M⁺Na]⁺: 493.1846; Found: 493.1845.

**tert-Butyl (3-(9-oxo-6-phenyl-7H-furo[3,2-e]pyrrolo[3,4-b]indolizin-8(9H)-yl)propyl)carbamate (03)**

Yield: 99%; ¹H NMR (400 MHz, CD₂Cl₂) δ 7.89 (d, J = 1.2 Hz, 1H), 7.68 (d, J = 1.6 Hz, 1H), 7.65 (d, J = 10.0 Hz, 1H), 7.57 (d, J = 8.4 Hz, 2H), 7.47 (t, J = 8.0 Hz, 2H), 7.33–7.27 (m, 2H), 5.50 (br s, 1H), 4.49 (s, 2H), 3.66 (t, J = 6.4 Hz, 2H), 3.13 (q, J = 6.1 Hz, 2H), 1.83–1.76 (m, 2H), 1.42 (s, 9H); ¹³C NMR (100 MHz, CD₂Cl₂) δ 162.2, 156.2, 144.0, 143.7, 135.1, 135.0, 134.6, 129.4, 127.7, 126.4, 126.2, 121.8, 113.8, 110.7, 110.5, 105.1, 79.0, 47.0, 40.4, 37.6, 29.1, 28.5; HRMS (ESI) m/z calcd for C₂₆H₂₇N₃NaO₄ [M⁺Na]⁺: 468.1894; Found: 468.1896.
**tert-Butyl (3-(6-(4-(diethylamino)phenyl)-9-oxo-7H-furo[3,2-e]pyrrolo[3,4-b]indolizin-8(9H)-yl)propyl)carbamate (04)**

Yield: 99%; ¹H NMR (400 MHz, acetone-\(d_6\)) \(\delta\) 7.90–7.88 (m, 2H), 7.67 (d, \(J = 9.6\) Hz, 1H), 7.45 (d, \(J = 9.2\) Hz, 2H), 7.34 (d, \(J = 9.6\) Hz, 1H), 6.81 (d, \(J = 8.8\) Hz, 2H), 6.14 (br s, 1H), 4.58 (s, 2H), 3.66 (t, \(J = 6.4\) Hz, 2H), 3.43 (q, \(J = 7.1\) Hz, 4H), 3.15 (q, \(J = 6.4\) Hz, 2H), 1.90–1.83 (m, 2H), 1.40 (s, 9H), 1.18 (t, \(J = 7.0\) Hz, 6H); ¹³C NMR (100 MHz, acetone-\(d_6\)) \(\delta\) 162.2, 156.6, 147.2, 144.7, 144.0, 134.7, 129.2, 126.2, 122.1, 122.0, 114.9, 113.1, 112.1, 109.8, 105.3, 78.5, 47.2, 44.9, 40.9, 38.3, 29.3, 28.6, 13.0; HRMS (ESI) m/z calcd for C\(_{30}\)H\(_{37}\)N\(_4\)O\(_4\) [M+H]⁺: 517.2809; Found: 517.2809.

**Procedure for preparation of 18**

To a solution of 01 (1.0 equiv.) in dimethylformamide (0.1 M), were added 4-bromophenyl methyl sulfone (10.0 equiv.), bis(triphenylphosphine)palladium(II) dichloride (20 mol%), silver acetate (1.5 equiv.) and potassium acetate (3.0 equiv.), and the solution was stirred at 100 °C. When the reaction was completed checked by TLC, the reaction mixture was filtered through the short bed of silica gel and concentrated \textit{in vacuo}. The residue was purified by silica-gel flash column chromatography to afford 18.

**tert-Butyl (3-(2,6-bis(4-(methylsulfonyl)phenyl)-9-oxo-7H-furo[3,2-e]pyrrolo[3,4-b]indolizin-8(9H)-yl)propyl)carbamate (18)**

Yield: 26%; ¹H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.31 (s, 1H), 8.05–7.99 (m, 6H), 7.75–7.70 (m, 3H), 7.43 (d, \(J = 9.6\) Hz, 1H), 5.24 (br s, 1H), 4.57 (s, 2H), 3.73 (t, \(J = 6.6\) Hz, 2H), 3.23–3.18 (m, 2H), 3.13 (s, 3H), 3.11 (s, 3H), 1.92–1.89 (m, 2H), 1.45 (s, 9H); ¹³C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 161.7, 156.2, 152.7, 144.2, 140.3, 140.0, 137.7, 135.2, 135.0, 134.6, 128.5, 128.2, 127.7, 127.6, 125.2, 122.8, 114.3, 111.0, 109.4, 102.2, 79.4, 47.0, 44.8, 44.7, 40.6, 37.7, 29.1, 28.6; HRMS (ESI) m/z calcd for C\(_{34}\)H\(_{35}\)N\(_3\)NaO\(_8\)S\(_2\) [M+Na]⁺: 700.1758; Found: 700.1759.
Procedure for preparation of Mito-18

To a solution of compound 18 in DCM (0.1 M) was added a 10% v/v of hydrochloric acid (HCl), and the solution was stirred overnight at room temperature. After full consumption of starting material, the solution was diluted with DCM and washed with sodium bicarbonate solution. The organic layer was extracted with DCM, dried over sodium sulfate and filtered through a cotton plug. The resulting solution was evaporated under reduced pressure to afford 18-1. LRMS (ESI) m/z calcd for C_{29}H_{28}N_{3}O_{6}S_{2} [M+H]^+: 578.14; Found : 578.1.

After that, 6-Bromohexanoyl chloride (1.5 equiv.) was slowly added to a stirred solution of 18-1 with TEA (3.0 equiv.) in DCM (0.1M) at room temperature and the solution was stirred for 6 h. After 18-1 was fully consumed, the solvent was removed, and crude mixture was dissolved in acetonitrile (0.1 M) followed by addition of triphenylphosphate (3.0 equiv.). The mixture was stirred under reflux condition for 2 days. The crude product was purified by reverse-phase-HPLC to afford a desired Mito-18. [HPLC solvents consist of water containing 0.1% TFA (trifluoroacetic acid) for solvent A, and acetonitrile containing 0.1% TFA for solvent B]

(6-((3-(2,6-bis(4-(methylsulfonyl)phenyl)-9-oxo-7H-furo[3,2-e]pyrrolo[3,4-b]indolizin-8(9H)-yl)propyl)amino)-6-oxohexyl)triphenylphosphonium (Mito-18)

Yield: 24% (overall yield over 3 steps); ^1H NMR (400 MHz, DMSO-d$_6$) δ 8.26 (s, 1H), 8.08 (d, $J = 8.4$ Hz, 2H), 8.04 (d, $J = 8.4$ Hz, 2H), 7.94–7.84 (m, 7H), 7.80–7.69 (m, 13H), 4.73 (s, 2H), 3.61–3.51 (m, 4H), 3.29 (s, 3H), 3.27 (s, 3H), 3.13–3.08 (q, $J = 6.5$ Hz, 2H), 2.06–2.03 (m, 2H), 1.81–1.76 (m, 2H), 1.51–1.47 (m, 6H); LRMS (ESI) m/z calcd for C$_{53}$H$_{51}$N$_3$O$_7$PS$_2$ [M]^+: 936.29; Found : 936.2.
4. Experimental Procedure for Live Cell Fluorescence Image

1. Cell culture

HeLa cell line (human cervical carcinoma cells) was obtained from American Type Culture Collection (ATCC). HeLa cells were cultured in RPMI 1640 (GIBCO) supplemented with heat-inactivated 10% (v/v) fetal bovine serum (FBS, GIBCO) and 1% (v/v) antibiotic-antimycotic agent (GIBCO). HeLa cell line was maintained in humidified atmosphere of 5% CO₂ and 95% air at 37 °C, and cultured in 100 mm cell culture dish (CORNING).

2. Mitochondria staining experiment with Mito-18 and MitoTracker Red

HeLa cells were seeded on cover glass bottom dish and incubated at 5 % CO₂, 37 °C for overnight. Cells are treated with 20 µM Mito-18 in media for 1 h. After 1 h, 20 nM MitoTracker Red CMXRos (Life Technologies) was added to cells, and incubate for 30 min. After the treatment, dyes were washed with PBS buffer for 3 times and then fluorescence images were taken by fluorescence microscopy under PBS buffer with DeltaVision Elite imaging system (GE Healthcare) equipped with 60X/1.42 NA oil lens. Fluorescence signal of each probes were obtained using FITC filter (Mito-18, Ex; 475 nm with 28 nm bandwidth, Em; 525 nm with 48 nm bandwidth) and Cy5 filter (MitoTracker Red CMXRox, Ex; 632 nm with 22 nm bandwidth, Em; 670 nm with 34 nm bandwidth).
5. Absorption and Emission Spectra of All furoindolizine Compounds

- Horizontal axis: wavelength (nm)
- Vertical axis: normalized intensity (a.u.)

**Blue line:** absorption spectra  
**Red line:** emission spectra
### 6. Computational Results of Furoindolizine Analogues

| # cpd | Electronic transition | Energy (eV) | (oscillator strength) |
|-------|------------------------|-------------|-----------------------|
| 01    | S₀ → S₁                | 3.7316 eV 332.26 nm | f = 0.2348 |
|       | S₀ → S₂                | 4.1879 eV 296.06 nm | f = 0.0420 |
|       | S₀ → S₃                | 4.7567 eV 260.65 nm | f = 0.0001 |
| 02    | S₀ → S₁                | 3.3275 eV 372.60 nm | f = 0.5467 |
|       | S₀ → S₂                | 3.7156 eV 333.68 nm | f = 0.0712 |
|       | S₀ → S₃                | 4.0517 eV 306.00 nm | f = 0.0142 |
| 03    | S₀ → S₁                | 3.4905 eV 355.21 nm | f = 0.3259 |
|       | S₀ → S₂                | 4.0271 eV 307.87 nm | f = 0.0706 |
|       | S₀ → S₃                | 4.2297 eV 293.13 nm | f = 0.1535 |
| 04    | S₀ → S₁                | 3.2084 eV 386.44 nm | f = 0.2737 |
|       | S₀ → S₂                | 3.8527 eV 321.81 nm | f = 0.0593 |
|       | S₀ → S₃                | 4.0629 eV 305.16 nm | f = 0.0159 |
| 05    | S₀ → S₁                | 2.9199 eV 424.62 nm | f = 0.6262 |
|       | S₀ → S₂                | 3.8066 eV 325.71 nm | f = 0.1642 |
|       | S₀ → S₃                | 4.0519 eV 305.99 nm | f = 0.0621 |
| 06    | S₀ → S₁                | 2.8076 eV 441.61 nm | f = 0.9179 |
|       | S₀ → S₂                | 3.4494 eV 359.43 nm | f = 0.2282 |
|       | S₀ → S₃                | 3.7989 eV 326.37 nm | f = 0.0862 |
| 07    | S₀ → S₁                | 2.7515 eV 450.61 nm | f = 0.7117 |
|       | S₀ → S₂                | 3.7433 eV 331.21 nm | f = 0.1957 |
|       | S₀ → S₃                | 3.8626 eV 320.99 nm | f = 0.1392 |
| 08    | S₀ → S₁                | 2.4405 eV 508.02 nm | f = 0.5137 |
|       | S₀ → S₂                | 3.2408 eV 382.57 nm | f = 0.3306 |
|       | S₀ → S₃                | 3.5917 eV 345.20 nm | f = 0.1929 |
| 09    | S₀ → S₁                | 3.2300 eV 383.85 nm | f = 0.5929 |
|       | S₀ → S₂                | 3.9430 eV 314.44 nm | f = 0.0602 |
|       | S₀ → S₃                | 4.3047 eV 288.02 nm | f = 0.0179 |
| 10    | S₀ → S₁                | 3.0152 eV 411.20 nm | f = 0.9641 |
|       | S₀ → S₂                | 3.4429 eV 360.11 nm | f = 0.0539 |
|       | S₀ → S₃                | 3.8750 eV 319.96 nm | f = 0.0503 |
| 11    | S₀ → S₁                | 3.0455 eV 407.11 nm | f = 0.6955 |
|       | S₀ → S₂                | 3.8669 eV 320.63 nm | f = 0.0636 |
|       | S₀ → S₃                | 3.9850 eV 311.12 nm | f = 0.2064 |
| 12    | S₀ → S₁                | 2.8039 eV 442.18 nm | f = 0.5454 |
|       | S₀ → S₂                | 3.6142 eV 343.05 nm | f = 0.2764 |
|       | S₀ → S₃                | 3.7556 eV 330.13 nm | f = 0.0947 |
Prediction of $\lambda_{em}$ and $\varepsilon$ for 17, 18 and 19

1. From Figure 3(f)

The equation for $\lambda_{em}$ estimation = 1092.9$x$ + 73.263, ($x$: calculated 1/eV)

2. From Figure S4

The equation for $\varepsilon$ estimation = 30382$y$ + 5221.8 ($y$: calculated oscillator strength values)

|     | $x$ (Calculated 1/eV) | Estimated $\lambda_{em}$ | $y$ (calculated oscillator strength values for $S_0\rightarrow S_1$) | Estimated $\varepsilon$ |
|-----|---------------------|---------------------------|-----------------------------------------------------------------|------------------------|
| 17  | 0.357935            | 464 nm                    | 0.9272                                                          | 33,392                 |
| 18  | 0.406835            | 518 nm                    | 0.8972                                                          | 32,480                 |
| 19  | 0.456496            | 572 nm                    | 0.6753                                                          | 25,738                 |
7. Copies of $^1$H and $^{13}$C NMR Spectra of all New Compounds
Sample 1: Operation: aliphatic

Details:
- Delay: 1.000 sec
- Pulse: 45.0 degrees
- Acquisition Time: 2.750 sec
- Window: 641.3 Hz
- Number of repetitions: 1000

Data Processing:
- FT size: 65536
- Total time: 5 minutes 30 seconds

Chemical Structure:

![Chemical Structure](image)

NMR Spectra:

![Spectra](image)
8. References

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