

Synthesis and properties of fluorescent 1,3-substituted mono and biindolizines

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Abstract

Substituted mono and biindolizines were synthesized in good yields via 3+2 dipolar cycloaddition of pyridinium ylides generated *in situ* from suitable 1-(2-oxoethyl)pyridinium bromides. The route allows the introduction a variety of substituents at positions 1 and 3 (1' and 3' for biindolizines) of the indolizine ring. All the synthesized compounds display pronounced fluorescence and detailed studies of the fluorescence properties in different solvents, as well as in acidic conditions for compounds **3a-e**, were investigated.

Keywords: Pyridinium ylides, 3+2 cycloaddition, indolizines, fluorescence

Introduction

Indolizine is an aromatic bicyclic 10 π -electron system and constitutional isomer of 1*H*-indole which, due to its special electronic structure, has long drawn much theoretical interest.^{1,2} Additionally, indolizine derivatives have been found to possess a variety of biological activities such as anti-inflammatory,³ antiviral,⁴ analgesic⁵ and antitumor⁶ activities. All these make indolizines an important synthetic target in view of developing new pharmaceuticals for the treatment of cancer,⁶ cardiovascular diseases,^{3b,7} and HIV infections.⁸ In addition, polycyclic indolizine and biindolizine derivatives have been found to have long wavelength absorption and strong fluorescence in the visible region.⁹ Considering these important properties and the steadily increasing importance of fluorophores in biolabeling and environmental trace analysis, the synthesis of these types of compounds has drawn the interest of chemists to develop new drugs, novel classes of fluorescent dyes or biological markers.

However, investigation of the biological activities, fluorescence and electric properties of indolizines is still impeded by the lack of efficient and general synthetic methods to synthesize indolizines with various functional groups at specific positions. Two general routes for indolizine

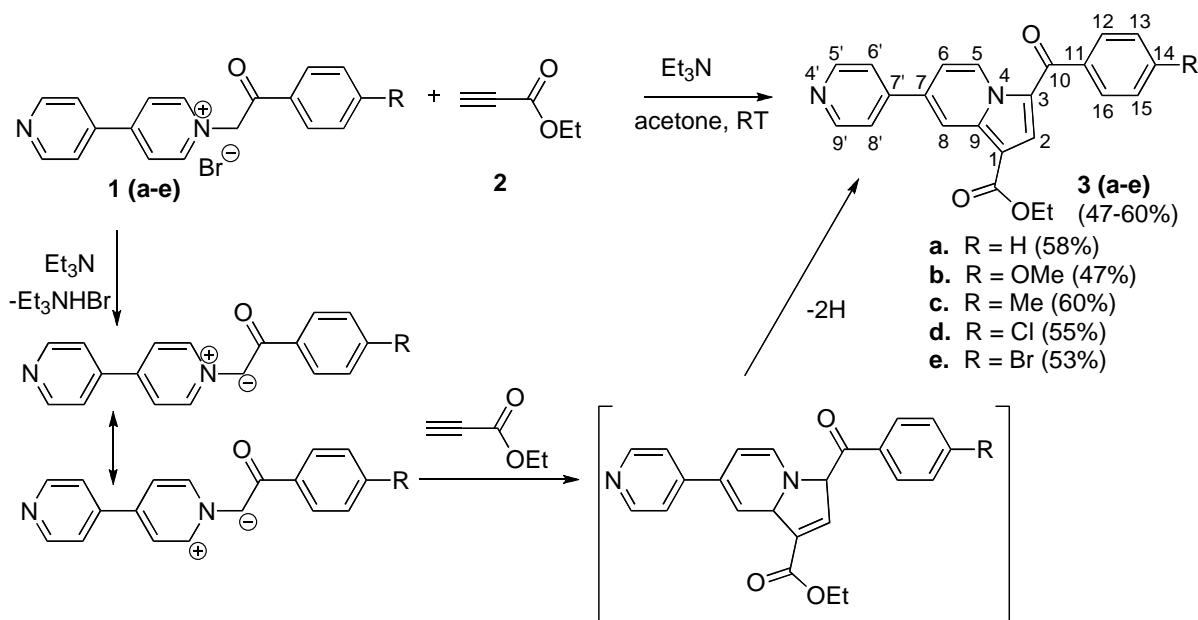
syntheses are known.^{2a} The first is based on the cyclizing condensation of suitable pyridinium precursors. The second approach takes advantage of a [3+2] cycloaddition of pyridinium ylides with various double or triple bond Michael acceptors.^{10,11}

Earlier, our group reported the synthesis of symmetrical and highly fluorescent biindolizines by the reaction of *in situ* generated symmetrical bis-ylides with activated alkynes.^{12a} Unsymmetrical indolizines were also synthesized by our group using unsymmetrical bis-ylides.^{12c} Use of these methods in labeling was limited, due to the symmetrical design and the impossibility of having only one labeled group. Here we report the synthesis and fluorescent properties of new substituted monoindolizines by [3+2] cycloaddition of pyridinium ylides with various activated alkynes as well as a new synthesis pathway to new unsymmetrically substituted biindolizines from monoindolizines, in order to increase the degree of asymmetry and also to investigate the possibility of introducing various substituents into the biindolizine ring.

Results and Discussion

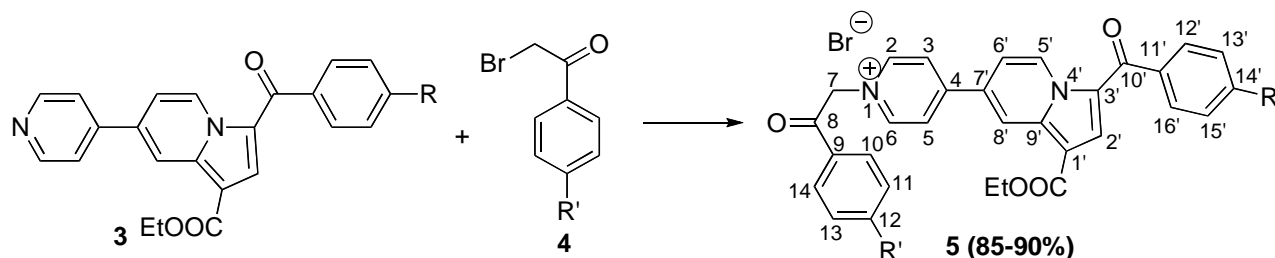
Synthesis

The synthesis and characterization of pyridinium monoquaternary salts of 4,4'-bipyridine were reported earlier by our group.^{12c,d} Such quaternary salts were used as starting materials for the indolizine syntheses described in this paper. First, the reactions of the *N*-ylides derived from the quaternary pyridinium salts **1**, with ethyl propiolate **2** were investigated (Scheme 1). Thus, 1-(2-oxoethyl)pyridinium bromide **1** (1.0 mmol) was deprotonated with triethylamine (3.0 mmol) to give the resonance stabilized pyridinium ylide *in situ*, an allyl-type 1,3-dipole, that readily underwent 1,3-dipolar cycloaddition with **2** (1.0 mmol) to give an intermediate dihydroindolizine as the expected cycloadduct. In agreement with the literature,^{9f,12} only indolizines **3** were isolated as a consequence of oxidative aromatization. These products are fully characterized by spectral (IR, ¹H and ¹³C NMR, and MS) and analytical data. Notably, the ¹H NMR spectra of **1a-e** were characterized by protons of the ester group (a triplet at $\delta = 1.34 - 1.44$ ppm and a quartet at $\delta = 3.85 - 4.42$) and an unusually low field absorption ($\delta = 9.95 - 10.03$ ppm) of the proton at C5 of indolizine ring caused by the strong anisotropic deshielding effect of the nearby carbonyl group.



Scheme 1. Synthesis of 7-pyridylindolizines by 3+2 dipolar cycloaddition of *in situ* generated ylides with ethyl propiolate.

Secondly, the synthesis of highly substituted, unsymmetrical indolizines was studied (Scheme 2). The pyridyl-indolizine **3** (1 mmol) was subjected to the reaction with bromoacetophenones **4** (1.1 mmol) in anhydrous acetone to achieve quaternization of the pyridine giving the indoliziny-pyridinium salts **5** in very good yields (Table 1). These compounds were characterized by spectral (IR and ^1H) and analytical data. Proton NMR spectroscopy showed a characteristically low field absorption of an indolizine proton at C5' ($\delta = 9.56 - 10.05$ ppm) and of the two protons at positions 3 and 5 of the pyridine ring ($\delta = 9.37 - 9.46$ ppm). The methylene protons adjacent to the quaternary nitrogen, were also very deshielded, showing signals at $\delta = 7.14-7.29$ ppm. In the IR spectra there were three absorption bands corresponding to the three C=O groups: one for the conjugated ester at $1698-1707$ cm^{-1} and the other two for the ketone C=O bonds at $1641-1687$ cm^{-1} and $1608-1644$ cm^{-1} .



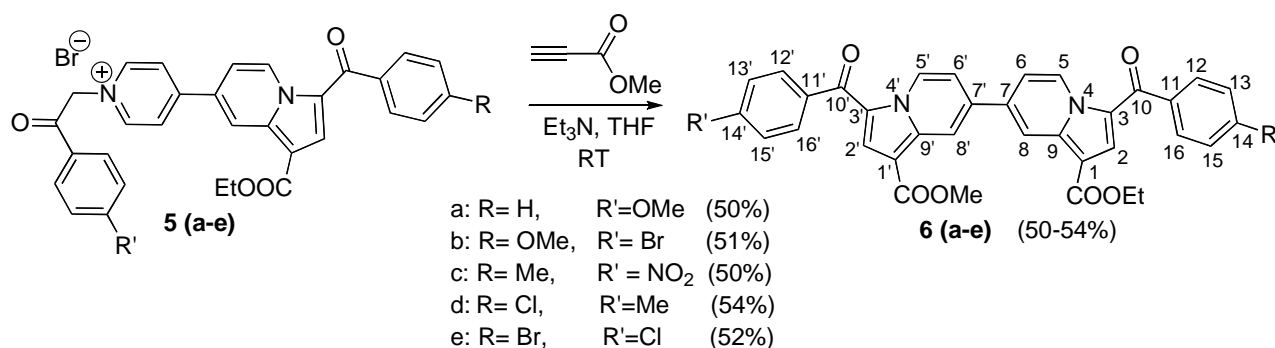
Scheme 2. Synthesis of indoliziny-pyridinium quaternary salts by quaternization with bromoacetophenone.

Table 1. Synthesis of indoliziny-pyridinium quaternary salts **5a-e**

Indolizine 3	Bromoacetophenone 4	Indoliziny-pyridinium salt 5 (yield) ^a
3 a , R = H	4 a , R' = OMe	5 a , (88%)
3 b , R = OMe	4 b , R' = Br	5 b , (85%)
3 c , R = Me	4 c , R' = NO ₂	5 c , (87%)
3 d , R = Cl	4 d , R' = Me	5 d , (90%)
3 e , R = Br	4 e , R' = Cl	5 e , (86%)

^a Isolated yields after column chromatography.

Using the indoliziny-pyridinium quaternary salts **5**, five new unsymmetrical biindolizines were synthesized using methyl propiolate (Scheme 3). Thus, compounds **5** (1.0 mmol) were suspended in THF together with equivalent amount of methyl propiolate (1.0 mmol) and then triethylamine (3.0 mmol) was added dropwise. As in the mechanism shown in Scheme 1, methyl propiolate reacted with *in situ* generated pyridinium ylides to give in good yields of the biindolizines **6** through oxidative aromatization of the intermediate dihydroindolizine. The ¹H-NMR spectra of the biindolizines showed signals for the new methyl ester group as singlets at 3.95 ppm and integration of the indolizine protons showed the presence of two such units. The IR spectra of these compounds showed absorption bands for the ester and ketone C=O groups.



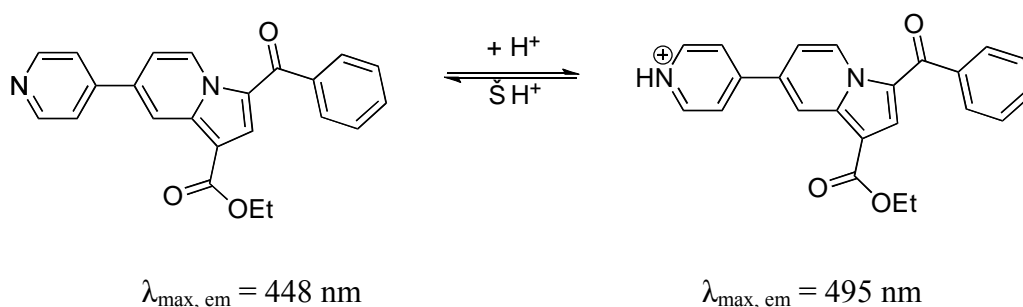
Scheme 3. Synthesis of unsymmetrical biindolizines by 3+2 dipolar cycloaddition of *in situ* generated indoliziny-pyridinium ylides with methyl propiolate.

Fluorescence measurements

As shown in previous studies^{9d-f} some indolizines and biindolizines with related structure are highly fluorescent (some of the biindolizines having a remarkably high quantum yield).^{9d,e} Thus the UV/VIS and fluorescence properties of compound **3a-e** in several solvents were investigated. Absorption maxima for all investigated compounds do not show big differences in cyclohexane, toluene, dichloromethane or ethanol, varying between 376 and 384 nm. In fluorescence spectra, maxima for compounds **3a-e** vary between 443 and 452 nm with a characteristic shoulder around 510 nm (Figure 1, blue curve).

The presence of a basic pyridine nitrogen atom in the 7-pyridyl indolizines **3a-e** suggested that there might be a pH-dependence of the emission properties similar to previously reported compounds.^{9f} To investigate the influence of the pyridine nitrogen atom, compound **3a** was dissolved in dichloromethane and the emission spectrum was recorded. Then, an excess of concentrated hydrochloric acid was added then the emission spectrum was recorded again (Figure 1, red curve). After protonation, a shift of the fluorescence maxima into the yellow region of the spectrum and a significant increase of fluorescence was observed.

To characterize compounds **3a-e** on the basis of absorption-emission properties, Stokes shifts for both non-protonated and protonated forms were calculated (Table 2). The data show slightly higher Stokes shifts in the protonated form in comparison to the non-protonated species, for all the investigated compounds.



Schematic representation of equilibrium of protonation-deprotonation for the compound **3a**.

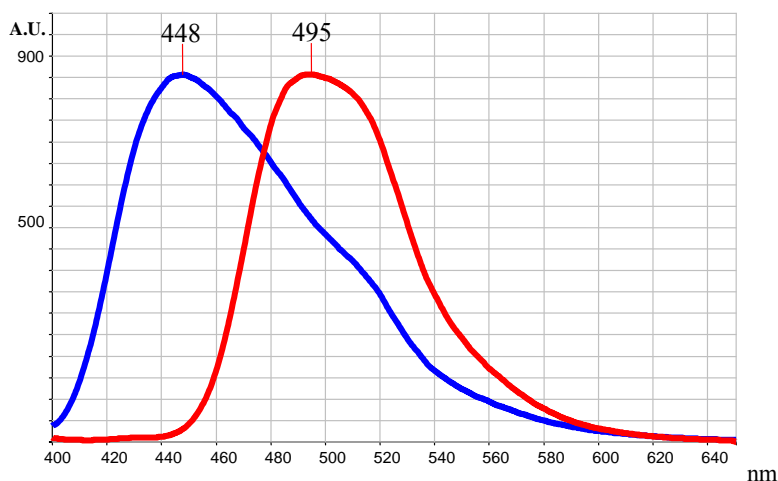


Figure 1. Fluorescence spectra of compound **3a** in dichloromethane (blue curve) and mixture of dichloromethane and concentrated hydrochloric acid (red curve) with the emission maxima at 448 nm for non protonated form and 495 nm for the protonated form.

Table 2. Maxima of absorption and emission and Stokes shifts, calculated for non-protonated and protonated forms of compounds **3a-e** in dichloromethane

Com pound	Absorption (nonprotonated) $\lambda_{\max, \text{abs}}$ [nm]	Absorption (protonated) $\lambda_{\max, \text{abs}}$ [nm]	Emission (nonprotonated) $\lambda_{\max, \text{em}}$ [nm]	Emission (protonated) $\lambda_{\max, \text{em}}$ [nm]	Stokes shift, ^a nonprotonated/ protonated $\Delta\tilde{\nu}$ [cm ⁻¹]
3 a	382	-	448	495	3860
3 b	381	419	443	500	3680/3870
3 c	383	413	449	496	3840/4050
3 d	384	412	447	494	3670/4030
3 e	386	410	451	492	3730/4070

^a $\Delta\tilde{\nu} = 1/\lambda_{\max, \text{abs}} - 1/\lambda_{\max, \text{em}}$ [cm⁻¹]. Values calculated by this equation are apparent and could give errors of 5-20%.¹³

Absorption and emission spectra for compounds **6a-e** were measured in dichloromethane (Figure 2) and the results are presented in Table 2. The fluorescence spectra showed maxima around 465 nm which is similar to previously reported biindolizines.^{9d-f} Further investigation of the solvent effects, quantum yields and influences of different substituents in the mono and biindolizine systems are underway.

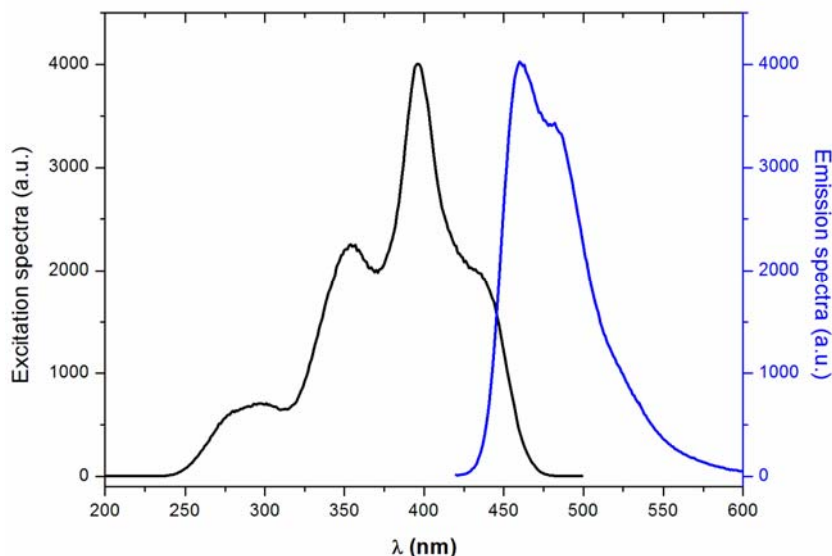
**Figure 2.** Absorption spectrum (black curve) with maximum at 399 nm and emission spectra (blue curve) with maximum at 460 nm of compound **6a** in dichloromethane.

Table 2. Maxima of absorption and emission and Stokes shifts, calculated for non-protonated and protonated forms of compounds **3a-e** in dichloromethane.

Com pound	Absorption $\lambda_{\max, \text{abs}}$ [nm]	Emission $\lambda_{\max, \text{em}}$ [nm]	Stokes shift, ^a $\Delta\tilde{\nu}$ [cm^{-1}]
6a	399	460	3860
6b	409	475	3680/3870
6c	407	465	3840/4050
6d	396	460	3670/4030
6e	402	465	3730/4070

$$^a \Delta\tilde{\nu} = 1/\lambda_{\max, \text{abs}} - 1/\lambda_{\max, \text{em}} [\text{cm}^{-1}].$$

In conclusion, we have successfully synthesized monoindolizines and biindolizines in good yields using 3+2 dipolar cycloadditions of pyridinium ylides generated *in situ* from suitable 1-(2-oxoethyl)pyridinium bromides. The route allows the introduction of substituents at positions 1 and 3 (1' and 3' for biindolizines) of the indolizine ring. All the new synthesized compounds displayed pronounced fluorescence and detailed studies of the fluorescence properties in dichloromethane for compounds **3a-e** and **6a-e** were made. Further studies will be mostly directed to investigations of the fluorescence and electrical properties of all the synthesized compounds in view of their direct relevance in biomolecule labeling and/or in the semiconductors field.

Experimental Section

General. Melting points were recorded on a Meltemp II melting point apparatus and are uncorrected. Proton and carbon nuclear magnetic resonance (δ_{H} , δ_{C}) spectra were recorded on Bruker Avance 400 DRX or Bruker ARX 300 spectrometers. All chemical shifts are quoted on the δ -scale in ppm using TMS or residual solvents (CHCl_3 , DMSO) as internal standards. Coupling constants are given in Hz. IR spectra were recorded on a FTIR Shimadzu spectrometer. Low resolution mass spectra were recorded on a Finnigan MAT 90 and MAT 95 Q; 70 eV, FAB in the positive mode; m/z (rel. int. in %). Thin layer chromatography (TLC) was carried out on Merck silica gel 60F₂₅₄ plates. Visualisation of the plates was achieved using a UV lamp ($\lambda_{\max} = 254$ or 365 nm). UV/vis spectra were measured using Perkin Elmer Models Lambda 16; Fluorescence spectra with Perkin Elmer LS-55 and spectrofluorimeter SLM-8000. Compounds **3a-e** and **6a-e** were dissolved in dichloromethane to the concentration of 10 μM and measured in 1 cm fluorescence cuvettes.

General procedure for preparation of 3a-e compounds

The cycloimmonium salt **1a-e** (1 mmol, 1 equiv., 0.36 g **1a**, 0.39 g **1b**, 0.37 g **1c**, 0.39 g **1d**, 0.43 g **1e**) and ethyl propiolate (1.1 mmol, 1.1 equiv., 0.11 g) were added to 10 mL of anhydrous acetone and the obtained suspension stirred at room temperature. A solution of triethylamine (TEA) (3 mmol, 3 equiv., 0.30 g) in anhydrous acetone (3 mL) was added dropwise over 2 h (magnetic stirring) and the resulting mixture was then stirred for 5-6 h at rt. Water (10 mL) was added and after stirring for another 15 minutes, the solid was collected by filtration to give a powder which was washed with 10 mL methanol. The product was recrystallized from ethanol-chloroform (1:1, v/v).

General procedure for synthesis of quaternary salts **5a-e**

A solution of monoindolizine **3** (1 mmol, 1 equiv., 0.37 g **3a**, 0.40 g **3b**, 0.38 g **3c**, 0.40 g **3d**, 0.45 g **3e**) and 2-bromo-4'-X-acetophenone (2 mmol, 2 equiv.) in anhydrous acetone (20 mL) was magnetically stirred for 3-4 h at reflux. The resulting bright yellow precipitate was removed by filtration and then washed with acetone. All products were purified by flash chromatography (DCM:MeOH 9:1, v/v).

General procedure for synthesis of biindolizines **6a-e**

The quaternary salt **5** (1 mmol, 1 equiv., 0.60 g **5a**, 0.68 g **5b**, 0.63 g **5c**, 0.62 g **5d**, 0.68 g **5e**) and methyl propiolate (1.1 mmol, 1.1 equiv., 0.09 g) were added to 40 mL of anhydrous tetrahydrofuran (THF) and the obtained suspension stirred at room temperature. A solution of triethylamine (TEA) (3 mmol, 3 equiv., 0.30 g) in anhydrous tetrahydrofuran (3 mL) was added dropwise over 2 h (magnetic stirring) and the resulting mixture was then stirred for 5-6 h at rt. Water (15 mL) was added and after stirring for another 15 min, the solid was collected by filtration to give a powder which was washed with 10 mL methanol. The product was recrystallized from ethanol-chloroform (1:4, v/v).

Ethyl 3-benzoyl-7-(pyridin-4-yl)indolizine-1-carboxylate 3a. Yellowish crystals (0.21g, 58% yield), mp 191–192 °C. ¹H-NMR (CDCl₃, 300 MHz): 9.95 (1H, d, H-5, *J* = 7.2 Hz), 8.65–8.69 (3H, m, H-5', H-9', H-8), 7.75–7.78 (3H, m, H-12, H-16, H-2), 7.58 (2H, d, H-6', H-8', *J* = 5.7 Hz), 7.44–7.54 (3H, m, H-13, H-14, H-15), 7.28 (1H, dd, H-6, *J* = 2.1, 7.5 Hz), 4.32 (2H, q, CH₂, *J* = 7.2 Hz), 1.35 (3H, t, CH₃, *J* = 7.2 Hz). ¹³C-NMR (CDCl₃, 75 MHz): 14.5 (CH₃), 60.4 (CH₂), 108.1 (C_{quat}), 113.2 (CH), 118.0 (CH), 122.2 (CH), 123.1 (C_{quat}), 128.5 (CH), 128.9 (CH), 129.1 (CH), 129.7 (CH), 131.9 (CH), 139.2 (C_{quat}), 139.4 (C_{quat}), 147.4 (CH), 163.7 (C_{quat}), 185.77 (C_{quat}). MS (70 eV, FAB⁺): *m/z* (%) 371 (M+H, 100), 370 (M⁺, 28). IR (KBr, ν(cm⁻¹)): 1689, 1609, 1287, 1199, 1134, 1078. Anal. Calcd. for C₂₃H₁₈N₂O₃: C, 74.58; H, 4.90; N, 7.56. Found: C, 74.66; H, 4.97; N, 7.75.

Ethyl 3-(4-methoxybenzoyl)-7-(pyridin-4-yl)indolizine-1-carboxylate 3b. Yellowish crystals (0.19g, 47% yield), mp 186–187 °C. ¹H-NMR (CDCl₃, 300 MHz): 9.89 (1H, d H-5, *J* = 7.5 Hz), 8.67–8.69 (3H, m, H-5', H-9', H-8), 7.78–7.81 (3H, m, H-12, H-16, H-2), 7.62 (2H, d, H-6', H-8', *J* = 5.7 Hz), 7.27 (1H, dd, H-6, *J* = 2.1, 7.5 Hz), 6.97 (2H, d, H-13, H-15, *J* = 8.7 Hz), 4.34 (2H, q, CH₂, *J* = 6.9 Hz), 3.85 (3H, s, OCH₃), 1.36 (3H, t, CH₃, *J* = 6.9 Hz). ¹³C-NMR (CDCl₃,

75 MHz): δ = 14.6 (CH₃), 55.5 (CH₃), 60.3 (CH₂), 107.1 (C_{quat}), 113.3 (CH), 113.8 (CH), 117.2 (CH), 121.2 (CH), 122.9 (C_{quat}), 128.5 (CH), 129.4 (CH), 131.3 (CH), 132.1 (C_{quat}), 136.4 (C_{quat}), 139.4 (C_{quat}), 145.3 (C_{quat}), 150.6 (CH), 162.7 (C_{quat}), 164.0 (C_{quat}), 184.6 (C_{quat}). MS (70 eV, FAB+): m/z (%) 401 (M+H, 100), 400 (M⁺, 32), 355 (M⁺-C₂H₅O), 328 (M⁺-C₃H₅O₂), 293 (M⁺-C₇H₇O). IR (KBr, ν (cm⁻¹): 1697, 1608, 1285, 1203, 1175, 1079. Anal. Calcd. for C₂₄H₂₀N₂O₄: C, 71.99; H, 5.03; N, 7.00. Found: C, 72.12; H, 5.21; N, 7.15.

Ethyl 3-(4-methylbenzoyl)-7-(pyridin-4-yl)indolizine-1-carboxylate 3c. Yellowish crystals (0.23g, 60% yield), mp 188–189 °C. ¹H-NMR (CDCl₃, 300 MHz): 10.02 (1H, dd, H-5, J = 0.6, 7.5 Hz), 8.75–8.78 (3H, m, H-5', H-9', H-8), 7.87 (1H, s, H-2), 7.78 (2H, d, H-12, H-16, J = 8.1 Hz), 7.68 (2H, d, H-6', H-8', J = 6.0 Hz), 7.35–7.38 (3H, m, H-13, H-15, H-6), 4.42 (2H, q, CH₂, J = 7.2 Hz), 2.49 (3H, s, CH₃), 1.44 (3H, t, CH₃, J = 7.2 Hz). ¹³C-NMR (CDCl₃, 75 MHz): δ = 14.5 (CH₃), 21.6 (CH₃), 60.2 (CH₂), 107.2 (C_{quat}), 113.4 (CH), 117.1 (CH), 121.2 (CH), 122.9 (C_{quat}), 128.9 (CH), 129.1 (CH), 129.5 (CH), 136.6 (C_{quat}), 136.8 (C_{quat}), 139.5 (C_{quat}), 142.4 (C_{quat}), 145.3 (C_{quat}), 150.6 (CH), 163.9 (C_{quat}), 185.5 (C_{quat}). MS (70 eV, FAB+): m/z (%) 385 (M+H, 100), 384 (M⁺, 38), 355 (M⁺-C₂H₅O), 328 (M⁺-C₃H₅O₂), 293 (M⁺-C₇H₇O). IR (KBr, ν (cm⁻¹): 1699 cm⁻¹, 1610, 1288, 1204, 1081, 1047. Anal. Calcd. for C₂₄H₂₀N₂O₃: C, 74.98; H, 5.24; N, 7.29. Found: C, 75.13; H, 5.32; N, 7.54.

Ethyl 3-(4-chlorobenzoyl)-7-(pyridin-4-yl)indolizine-1-carboxylate (3d). Yellowish crystals (0.22g, 55% yield), mp 215–216 °C. ¹H-NMR (CDCl₃, 300 MHz): 9.93 (1H, dd, H-5, J = 0.9, 7.5 Hz), 8.68–8.71 (3H, m, H-5', H-9', H-8), 7.71–7.75 (3H, m, H-12, H-16, H-2), 7.61 (2H, dd, J = 1.5, 7.5 Hz, H-6', H-8'), 7.45 (2H, d, H-13, H-15, J = 8.7 Hz), 7.31 (1H, dd, H-6, J = 1.8, 7.2 Hz), 4.34 (2H, q, CH₂, J = 6.9 Hz), 1.36 (3H, t, CH₃, J = 6.9 Hz). ¹³C-NMR (CDCl₃, 75 MHz): δ = 14.5 (CH₃), 60.4 (CH₂), 107.8 (C_{quat}), 113.6 (CH), 117.5 (CH), 121.8 (CH), 122.5 (C_{quat}), 126.7 (C_{quat}), 128.9 (CH), 129.6 (CH), 130.6 (CH), 131.7 (CH), 136.2 (C_{quat}), 138.1 (C_{quat}), 139.7 (C_{quat}), 146.6 (C_{quat}), 149.1 (CH), 163.8 (C_{quat}), 184.4 (C_{quat}). IR (KBr, ν (cm⁻¹): 1699 cm⁻¹, 1609, 1286, 1200, 1078, 1092. Anal. Calcd. for C₂₃H₁₇ClN₂O₃: C, 68.23; H, 4.23; N, 6.92; Found: C, 68.35; H, 4.38; N, 7.11.

Ethyl 3-(4-bromobenzoyl)-7-(pyridin-4-yl)indolizine-1-carboxylate (3e). Yellowish crystals (0.24g, 53% yield), mp 234–235 °C. ¹H-NMR (CDCl₃, 300 MHz): 10.01 (1H, d, H-5, J = 7.2 Hz), 8.77–8.79 (3H, m, H-5', H-9', H-8), 7.82 (1H, s, H-2), 7.66–7.75 (6H, m, H-13, H-15, H-6', H-8', H-12, H-16), 7.39 (1H, dd, H-6, J = 2.1, 7.5 Hz), 4.42 (2H, q, CH₂, J = 7.2 Hz), 1.44 (3H, t, CH₃, J = 7.2 Hz). ¹³C-NMR (CDCl₃, 75 MHz): δ = 14.5 (CH₃), 60.4 (CH₂), 107.9 (C_{quat}), 113.5 (CH), 117.5 (CH), 121.7 (CH), 122.5 (C_{quat}), 126.6 (C_{quat}), 128.9 (CH), 129.5 (CH), 130.5 (CH), 131.7 (CH), 136.1 (C_{quat}), 138.1 (C_{quat}), 139.6 (C_{quat}), 146.6 (C_{quat}), 149.1 (CH), 163.7 (C_{quat}), 184.3 (C_{quat}). MS (70 eV, FAB+): m/z (%) 449/451 (M+H, 98/100), 448/450 (M⁺, 23/40), 403/405 (M⁺-C₂H₅O, 12/11). IR (KBr, ν (cm⁻¹): 1700 cm⁻¹, 1610, 1288, 1201, 1076, 1076. Anal. Calcd. for C₂₃H₁₇BrN₂O₃: C, 61.48; H, 3.81; N, 6.23; Found: C, 61.60; H, 3.96; N, 6.53.

1-[2-(4-Methoxyphenyl)-2-oxoethyl]-4-[[1-(ethoxycarbonyl)-3-benzoyl]-indolizin-7-yl]pyridinium bromide 5a. Bright yellow crystals (0.52g, 88% yield), mp 274–276 °C. ¹H-NMR: 9.56 (1H, d, H-5', J = 7.6Hz), 9.37 (2H, d, H-2, H-6, J = 6.4 Hz), 8.81 (1H, d, H-8', J = 2

Hz), 8.33 (2H, d, H-3, H-5, $J = 6.4$ Hz), 8.16 (2H, d, H-10, H-14, $J = 8.8$ Hz), 7.81 (3H, ad, H-12', H-16', H-2'), 7.63 (1H, t, H-14', $J = 7.6$ Hz), 7.53 (2H, t, H-13', H-15', $J = 7.6$ Hz), 7.46 (1H, dd, H-6', $J = 7.6$ Hz, $J = 2$ Hz), 7.15 (2H, s, H-7), 6.95 (2H, d, H-11, H-13, $J = 8.8$ Hz), 4.42 (2H, q, CH₂, $J = 7.2$ Hz), 3.84 (3H, s, OCH₃), 1.42 (3H, t, CH₃, $J = 7.2$ Hz). IR (KBr, $\nu(\text{cm}^{-1})$): 1706, 1689, 1643, 1241, 1205, 1178. Anal. Calcd. for C₃₂H₂₇BrN₂O₅: C, 64.11; H, 4.54; N, 4.67; Found: C, 64.30; H, 4.69; N, 4.79.

1-[2-(4-Bromophenyl)-2-oxoethyl]-4-[[1-(ethoxycarbonyl)-3-(4-methoxybenzoyl)]indolizin-7-yl]pyridinium bromide 5b. Yellow crystals (0.57g, 85% yield), m.p. 260-262 °C. ¹H-RMN: 9.77 (1H, d, H-5', $J = 7.6$ Hz), 9.46 (2H, d, H-3, H-5, $J = 6.8$ Hz), 8.2 (1H, as, H-8'), 8.35 (2H, d, H-2, H-6, $J = 6.8$ Hz), 8.00 (2H, d, H-10, H-14, $J = 8.8$ Hz), 7.78 (2H, d, H-12', H-16', $J = 8.8$ Hz), 7.72 (1H, s, H-2'), 7.51 (2H, d, H-11, H-13, $J = 8.8$ Hz), 7.47 (1H, dd, H-6', $J = 7.6$ Hz, $J = 2$ Hz), 7.28 (2H, s, H-7), 6.98 (2H, d, H-13', H-15', $J = 8.8$ Hz), 4.40 (2H, q, CH₂, $J = 7.2$ Hz), 3.91 (3H, s, OCH₃), 1.40 (3H, t, CH₃, $J = 7.2$ Hz). IR (KBr, $\nu(\text{cm}^{-1})$): 1699, 1641, 1608, 1257, 1201, 1170. Anal. Calcd. for C₃₂H₂₆Br₂N₂O₅: C, 56.66; H, 3.86; N, 4.13; Found: C, 56.80; H, 3.99; N, 4.59.

1-[2-(4-Nitrophenyl)-2-oxoethyl]-4-[[1-(ethoxycarbonyl)-3-(4-methylbenzoyl)]indolizin-7-yl]pyridinium bromide 5c. Yellow crystals (0.54g, 87% yield), m.p. 260-262 °C. ¹H-NMR: 9.91 (1H, d, H-5', $J = 7.2$ Hz), 9.40 (2H, d, H-3, H-5, $J = 6.4$ Hz), 8.85 (1H, d, H-8', $J = 1.8$ Hz), 8.35 (2H, d, H-2, H-6, $J = 6.4$ Hz), 8.11 (2H, d, H-11, H-13, $J = 8.2$ Hz), 7.87 (2H, d, H-12', H-16', $J = 8.7$ Hz), 7.83 (1H, s, H-2'), 7.65 (2H, d, H-10, H-14, $J = 8.2$ Hz), 7.44 (1H, dd, H-6', $J = 7.2$ Hz, $J = 1.8$ Hz), 7.29 (2H, s, H-7), 7.24 (2H, d, H-13', H-15', $J = 8.7$ Hz), 4.46 (2H, q, CH₂, $J = 7.2$ Hz), 2.48 (3H, s, CH₃), 1.49 (3H, t, CH₃, $J = 7.2$ Hz). IR (KBr, $\nu(\text{cm}^{-1})$): 1706, 1689, 1644, 1527, 1349, 1235, 1208, 1202. Anal. Calcd. for C₃₂H₂₆BrN₃O₆: C, 61.16; H, 4.17; N, 6.69; Found: C, 61.29; H, 4.09; N, 6.90.

1-[2-(4-Methylphenyl)-2-oxoethyl]-4-[[1-(ethoxycarbonyl)-3-(4-chlorobenzoyl)]indolizin-7-yl]pyridinium bromide 5d. Yellow crystals (0.55g, 90% yield), m.p. 268-280 °C. ¹H-NMR: 10.05 (1H, d, H-5', $J = 7.5$ Hz), 9.46 (2H, d, H-3, H-5, $J = 6.9$ Hz), 8.96 (1H, d, H-8', $J = 1.5$ Hz), 8.35 (2H, d, H-2, H-6, $J = 6.9$ Hz), 8.13 (2H, d, H-12', H-16', $J = 8.4$ Hz), 7.87 (1H, s, H-2'), 7.83 (2H, d, H-10, H-14, $J = 8.4$ Hz), 7.58 (2H, d, H-13', H-15', $J = 8.4$ Hz), 7.47 (1H, dd, H-6', $J = 7.5$ Hz, $J = 1.5$ Hz), 7.39 (2H, d, H-11, H-13, $J = 8.4$ Hz), 7.14 (2H, s, H-7), 4.48 (2H, q, CH₂, $J = 7.2$ Hz), 2.48 (3H, s, CH₃), 1.42 (3H, t, CH₃, $J = 7.2$ Hz). IR (KBr, $\nu(\text{cm}^{-1})$): 1698, 1610, 1207, 1081. Anal. Calcd. for C₃₂H₂₆BrClN₂O₄: C, 62.20; H, 4.24; N, 4.53; Found: C, 62.49; H, 4.59; N, 4.70.

1-[2-(4-Chlorophenyl)-2-oxoethyl]-4-[[1-(ethoxycarbonyl)-3-(4-bromobenzoyl)]indolizin-7-yl]pyridinium bromide 5e. Orange crystals (0.58g, 86% yield), m.p. 268-270 °C. ¹H-NMR: 9.97 (1H, d, H-5', $J = 7.5$ Hz), 9.41 (2H, d, H-3, H-5, $J = 6.6$ Hz), 8.88 (1H, d, H-8', $J = 1.2$ Hz), 8.37 (2H, d, H-2, H-6, $J = 6.6$ Hz), 8.18 (2H, d, H-10, H-14, $J = 8.7$ Hz), 7.80 (1H, s, H-2'), 7.72 (2H, d, H-12', H-16', $J = 7.5$ Hz), 7.48 (3H, ad, H-6', H-11, H-13), 7.30 (2H, d, H-13', H-15', $J = 7.5$ Hz), 7.29 (2H, s, H-7), 4.46 (2H, q, CH₂, $J = 7.2$ Hz), 1.44 (3H, t, CH₃, $J = 7.2$

Hz). IR (KBr, $\nu(\text{cm}^{-1})$): 1707, 1687, 1639, 1234, 1203, 1176. Anal. Calcd. for $\text{C}_{31}\text{H}_{23}\text{BrClN}_2\text{O}_4$: C, 54.53; H, 3.40; N, 4.10; Found: C, 54.49; H, 3.59; N, 4.40.

1-Ethyl 1'-methyl 3-benzoyl-3'-(4-methoxybenzoyl)-7,7'-biindolizine-1,1'-dicarboxylate 6a. Yellow crystals (0.30g, 50% yield), m.p. 293-294 °C. ^1H -RMN: 10.04, 9.97 (2H, 2d, H-5 H-5', $J = 7.2$ Hz), 8.79 (2H, as, H-8, H-8'), 7.86 (6H, aq, H-2, H-2', H-12, H-16, H-12', H-16'), 7.48-7.63 (5H, m, H-13, H-15, H-14, H-6, H-6'), 7.04 (2H, d, H-13', H-15' $J = 8.8$ Hz), 4.42 (2H, q, CH_2 , $J = 7.2$ Hz), 3.95 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 1.44 (3H, t, CH_3 , $J = 7.2$ Hz). IR (KBr, $\nu(\text{cm}^{-1})$): 1701, 1685, 1616, 1263, 1199, 1174. Anal. Calcd. for $\text{C}_{36}\text{H}_{28}\text{N}_2\text{O}_7$: C, 71.99; H, 4.70; N, 4.66; Found: C, 72.15; H, 4.69; N, 4.95.

1-Ethyl 1'-methyl 3-(4-methoxybenzoyl)-3'-(4-bromobenzoyl)-7,7'-biindolizine-1,1'-dicarboxylate 6b. Yellow crystals (0.34g, 51% yield), m.p. 350-352 °C. ^1H -RMN: 9.97 (2H, m, H-5, H-5'), 8.82 (2H, as, H-8, H-8'), 7.82-7.88 (3H, m, H-2, H-12', H-16') 7.72-7.66 (3H, m, H-2, H-12, H-16) 7.52 (2H, m, H-6, H-6'), 7.26 (2H, m, H-13', H-15'), 7.05 (2H, d, H-13, H-15, $J = 8.2$ Hz), 4.42 (2H, q, CH_2 , $J = 7.2$ Hz), 3.95 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 1.43 (3H, t, CH_3 , $J = 7.2$ Hz). IR (KBr, $\nu(\text{cm}^{-1})$): 1734, 1701, 1643, 1616, 1232, 1204, 1147, 1104. Anal. Calcd. for $\text{C}_{36}\text{H}_{27}\text{BrN}_2\text{O}_7$: C, 63.63; H, 4.00; N, 4.12; Found: C, 63.85; H, 4.09; N, 4.35.

1-Ethyl 1'-methyl 3-(4-methylbenzoyl)-3'-(4-nitrobenzoyl)-7,7'-biindolizine-1,1'-dicarboxylate 6c. Dark orange crystals (0.31g, 50% yield), m.p. 319-321 °C. ^1H -RMN: 10.03 (2H, at, H-5, H-5', $J = 7.6$ Hz), 8.83 (1H, s, H-8'), 8.82 (1H, s, H-8), 8.40 (2H, d, H-13', H-15', $J = 8.8$ Hz), 7.99 (2H, d, H-12', H-16', $J = 8.8$ Hz), 7.87 (1H, s, H-2), 7.78 (3H, ad, H-2, H-12, H-16), 7.59 (1H, dd, H-6', $J = 7.2$ Hz, $J = 1.6$ Hz), 7.49 (1H, dd, H-6, $J = 7.2$ Hz, $J = 1.6$ Hz), 7.35 (2H, d, H-13, H-15, $J = 8$ Hz), 4.43 (2H, q, CH_2 , $J = 7.2$ Hz), 3.95 (3H, s, OCH_3), 2.48, (3H, s, CH_3), 1.45 (3H, t, CH_3 , $J = 7.2$ Hz). IR (KBr, $\nu(\text{cm}^{-1})$): 1718, 1703, 1643, 1620, 1525, 1338, 1201, 1182, 1137, 1080. Anal. Calcd. for $\text{C}_{36}\text{H}_{27}\text{N}_3\text{O}_8$: C, 68.67; H, 4.32; N, 6.67; Found: C, 68.65; H, 4.39; N, 6.95.

1-Ethyl 1'-methyl 3-(4-chlorobenzoyl)-3'-(4-methylbenzoyl)-7,7'-biindolizine-1,1'-dicarboxylate 6d. Yellow crystals (0.33g, 54% yield), m.p. 327-329 °C. ^1H -RMN: 10.02, 10.01 (2H, 2d, H-5, H-5', $J = 5.2$ Hz), 8.8 (2H, as, H-8, H-8'), 7.87, 7.83 (2H, 2s, H-2, H-2'), 7.80 (2H, d, H-12', H-16', $J = 8.4$ Hz), 7.77 (2H, d, H-12, H-16, $J = 8$ Hz), 7.52 (4H, aq, H-6', H-6, H-13, H-15), 7.35 (2H, d, H-13', H-15', $J = 8.4$ Hz), 4.42 (2H, q, CH_2 , $J = 7.2$ Hz), 3.95 (3H, s, OCH_3), 2.48 (3H, s, CH_3), 1.44 (3H, t, CH_3 , $J = 7.2$ Hz). IR (KBr, $\nu(\text{cm}^{-1})$): 1705, 1622, 1217, 1083, 1147, 1045. Anal. Calcd. for $\text{C}_{36}\text{H}_{27}\text{ClN}_2\text{O}_6$: C, 69.85; H, 4.40; N, 4.53; Found: C, 69.73; H, 4.39; N, 4.80.

1-Ethyl 1'-methyl 3-(4-bromobenzoyl)-3'-(4-chlorobenzoyl)-7,7'-biindolizine-1,1'-dicarboxylate 6e. Yellow crystals (0.33g, 52% yield), m.p. 334-335 °C. ^1H -RMN: 10.01 (2H, d, H-5, H-5', $J = 7.2$ Hz), 8.83 (2H, ad, H-8, H-8'), 7.82-7.70 (8H, m, H-2, H-2', H-12, H-16, H-12', H-16', H-6, H-6'), 7.53 (4H, m, H-13, H-15, H-13', H-15'), 4.42 (2H, q, CH_2 , $J = 7.2$ Hz), 3.95 (3H, s, OCH_3), 1.43 (3H, t, CH_3 , $J = 7.2$ Hz). IR (KBr, $\nu(\text{cm}^{-1})$): 1705, 1622, 1217, 1083, 1147, 1045. Anal. Calcd. for $\text{C}_{35}\text{H}_{24}\text{BrClN}_2\text{O}_6$: C, 61.46; H, 3.54; N, 4.10; Found: C, 61.63; H, 3.40; N, 4.28.

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