

Synthesis and Characterization of Ethyl 7-Acetyl-2-substituted 3-(substituted benzoyl)indolizine-1-carboxylates for *in vitro* Anticancer Activity

C. SANDEEP^{1,2}, BASAVARAJ PADMASHALI^{1,3,*}, KATHARIGATTA N. VENUGOPALA^{4,*},
RASHMI S. KULKARNI⁵, RASHMI VENUGOPALA⁶ and BHARTI ODHAV⁴

¹Department of Chemistry, Sahyadri Science College (Autonomous), Shimoga-577 203, India

²Institute for Stem Cell Biology and Regenerative Medicine, NCBS, TIFR, GKVK, Bellary Road, Bangalore-560 065, India

³Department of Studies and Research in Chemistry, School of Basic Sciences, Rani Channamma University, Belagavi-591 156, India

⁴Department of Biotechnology and Food Technology, Durban University of Technology, Durban-4001, South Africa

⁵Department of Chemistry, Jain University, Bangalore-560 019, India

⁶Department of Public Health Medicine, University of KwaZulu-Natal, Howard College Campus, Durban-4001, South Africa

*Corresponding authors: Tel. +27 31 3736887; Fax: +27 86 2423534; E-mail: basavarajpadmashali@yahoo.com; katharigattav@dut.ac.za

Received: 25 August 2015;

Accepted: 17 November 2015;

Published online: 30 January 2016;

AJC-17744

Efficient synthesis of a series of novel indolizines (**2a-r**) has been achieved by reaction between 4-acetyl-1-[2-(substituted phenyl)-2-oxoethyl]pyridin-1-ium bromide and substituted alkynes in presence of anhydrous potassium carbonate in dimethyl formamide medium. The title compounds have been characterized by spectroscopic techniques and elemental analysis. Selected compounds **2b**, **2h**, **2i**, **2q** and **2r** have been screened for *in vitro* anticancer activity using adriamycin as positive control and it was found that compounds **2b**, **2q** and **2r** have shown significant anticancer activity.

Keywords: Anticancer, Characterization, Indolizine analogues, Synthesis.

INTRODUCTION

Indolizines are heteroaromatic compounds containing condensed five and six membered rings with bridging nitrogen. They are isoelectronic with indole and represent a group of heterocyclic compounds structurally related to purines. Indolizine skeletons with different degrees of unsaturation are present in wide variety of natural and unnatural azacyclic compounds. Most of the naturally occurring indolizines have been isolated from species of genus *dendrobates* (poison-arrow frogs) [1,2]; monomorium (ants) [3]; dendrobium (orchids) [4]; tylophora [5] and the leguminosae family (plants). Indolizine alkaloids display broad spectrum of biological activities. Polyhydroxylated indolizine alkaloids are excellent inhibitors of biologically important pathways. These include the binding and processing of glycoproteins [6], potent glycosidase inhibitor activities [7,8], activity against AIDS [9,10] as well as against other important pathologies [11]. The 1-azabicyclo[4.3.0]nonane (indolizine) framework occupies a special place in heterocyclic systems due to the presence of this structural assembly in a number of natural products of biological importance such as tabersonine [12], (-)-strychnine [13], (+)-vinblastine [14], (-)-monomorphine [15], (-)-gephyrotoxin

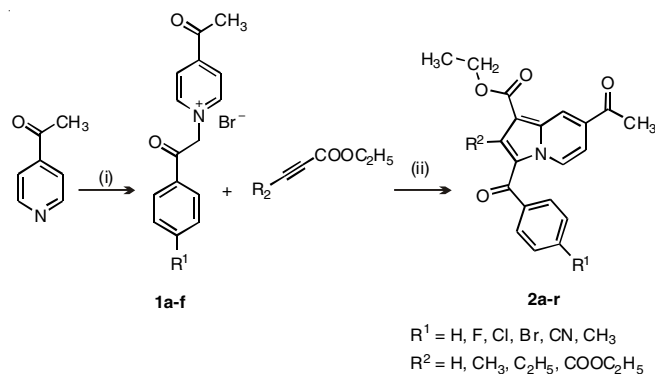
[16], etc. On the other hand, synthetic indolizine derivatives have been reported as calcium channel blockers [17], phospholipase A2 inhibitors [18], histamine H₃-receptors antagonist [19] and 5-HT₃-receptors antagonists [20]. Besides this, indolizines are also associated with pharmacological properties such as anti-inflammatory (cyclooxygenase inhibitors) [21,22], anti-tumour (alkylating agents) [23,24], oral hypoglycaemic [25] and CNS activities [26,27].

Because of unexceptional potential of these indolizines, noteworthy advances on their synthesis and biological evaluation have gone unreported. A careful look at the indolizine framework would logically suggest that one step or one-pot simultaneously tandem construction of the N-C bond and C-C bond on to six membered nitrogen heterocycles (piperidine/pyridine), in an appropriately organized manner using a suitable reagent would lead to the formation of the desired azabicyclo-(4.3.0)nonane frame work [28-32].

Typical molecular constructions of indolizines fall into three classes. (a) Condensation reactions of a 2-alkylpyridine with acid anhydrides (Scholta reaction) [33] or α -haloketones (Tschtischibabin reaction) [34-36]. (b) Reaction of an α -unsubstituted pyridine with a three carbon fragment such as an acyl or aryl substituted allyl halides or esters [37] and methyl

propiolates. (c) Reaction of pyridinium *N*-methylides generated from pyridinium salts under K_2CO_3 [38,39], pyridine and carbenes [40] or *N*-trimethylsilylmethylpyridinium triflates under fluoride ion [41] with acetylenes or reaction of pyridinium *N*-methylides with ethylene in the presence of an oxidant [42].

Keeping these observations in mind and in continuation of our research on pharmacologically active heterocyclic compounds [43,44] and polymorphism [45,46], herewith we undertake synthesis and characterization of ethyl 7-acetyl-3-(4-substituted benzoyl)-2-substituted indolizine-1-carboxylate (**2a-r**, **Scheme-I**) for *in vitro* anticancer properties.



Scheme-I: Reagents and conditions: (i) 4-substituted phenacyl bromide, acetone, 5 h, stir; (ii) K_2CO_3 , DMF, 0.5 h stir at room temperature

EXPERIMENTAL

Commercially available chemicals were procured from Sigma Aldrich. Hot-air dried glass wares were used to carry out reactions under nitrogen atmosphere using dry solvents. Monitoring of chemical reactions was done on analytical thin layer chromatography (TLC) with Merck 60 F-254 silica-gel plates. NMR spectra (1H and ^{13}C) were recorded at ambient temperature using $CDCl_3$, $DMSO-d_6$ as a solvent on 400 MHz Bruker-spectrometer using tetramethylsilane (TMS) as internal standard. Chemical shifts were showed in ppm (δ) and were referenced with TMS. LC-MS analysis was performed on Agilent LC-1200 series coupled with 6140 single quad mass spectro-meter with ESI +ve and -ve mode, MS range from 100-2000. IR spectra were recorded using KBr pellets on Bruker alpha FT-IR spectrometer. Perkin Elmer CHNS analyser was used for elemental analyses.

General procedure for the synthesis of 4-acetyl-1-(2-(4-substituted phenyl)-2-oxoethyl)pyridinium bromide (1a-f): To a stirred solution of 4-acetylpyridine (0.1 mol, 12.1 g) in dry acetone (60 mL), substituted phenacyl bromide (0.1 mol) was added and stirred at room temperature for 5 h. The reaction completion was monitored on TLC, product formed was filtered and dried under vacuum to afford compounds **1a-f**.

4-Acetyl-1-(2-oxo-2-phenylethyl)pyridiniumbromide (1a): White solid. Yield 99 %. 1H NMR (400 MHz, $DMSO-d_6$): δ 9.15 (d, $J = 8$ Hz, 2H), 8.60 (d, $J = 6.8$ Hz, 2H), 8.07-8.04 (m, 2H), 7.81-7.77 (m, 1H), 7.68-7.64 (m, 2H), 6.53 (s, 2H), 2.76 (s, 3H). LC-MS (ESI, Positive) m/z 240 $[M+H]^+$. Anal. calcd. for $C_{15}H_{14}BrNO_2$: C, 56.27; H, 4.41; N, 4.37 %. Found: C, 56.09; H, 4.39; N, 4.30 %.

4-Acetyl-1-[2-(4-fluorophenyl)-2-oxoethyl]pyridiniumbromide (1b): White solid. Yield 98 %. 1H NMR (400

MHz, $DMSO-d_6$): δ 9.22 (d, $J = 6.8$ Hz, 2H), 8.64 (d, $J = 7.2$ Hz, 2H), 8.20-8.16 (m, 2H), 7.54 (t, $J = 8.8$ Hz, 2H), 6.58 (s, 2H), 2.79 (s, 3H). LC-MS (ESI, Positive): m/z 258 $[M+H]^+$. Anal. calcd. for $C_{15}H_{13}BrFNO_2$: C, 53.27; H, 3.87; N, 4.14 %. Found: C, 53.03; H, 3.81; N, 4.05 %.

4-Acetyl-1-[2-(4-chlorophenyl)-2-oxoethyl]pyridiniumbromide (1c): Light yellow solid. Yield 98 %. 1H NMR (400 MHz, $DMSO-d_6$): δ 9.19 (d, $J = 7$ Hz, 2H), 8.64 (d, $J = 4$ Hz, 2H), 8.10 (d, $J = 7.4$ Hz, 2H), 7.78 (d, $J = 7.2$ Hz, 2H), 6.55 (s, 2H), 2.79 (s, 3H). LC-MS (ESI, Positive): m/z 274 $[M+H]^+$. Anal. calcd. for $C_{15}H_{13}BrClNO_2$: C, 50.80; H, 3.69; N, 3.95; %. Found: C, 50.70; H, 3.59; N, 3.90 %.

4-Acetyl-1-[2-(4-bromophenyl)-2-oxoethyl]pyridiniumbromide (1d): Yellow solid. Yield 99 %. 1H NMR (400 MHz, $DMSO-d_6$): δ 9.19 (d, $J = 7.2$ Hz, 2H), 8.63 (d, $J = 7.0$ Hz, 2H), 8.01 (d, $J = 7.2$ Hz, 2H), 7.91 (d, $J = 8$ Hz, 2H), 6.54 (s, 2H), 2.78 (s, 3H). LC-MS (ESI, Positive): m/z 318 $[M+H]^+$. Anal. calcd. for $C_{15}H_{13}Br_2NO_2$: C, 45.14; H, 3.28; N, 3.51 %. Found: C, 45.01; H, 3.18; N, 3.46 %.

4-Acetyl-1-[2-(4-cyanophenyl)-2-oxoethyl]pyridiniumbromide (1e): Light brown solid. Yield 99 %. 1H NMR (400 MHz, $DMSO-d_6$): δ 9.15 (d, $J = 7.2$ Hz, 2H), 8.61 (d, $J = 7.0$ Hz, 2H), 7.99 (d, $J = 7.2$ Hz, 2H), 7.87 (d, $J = 8$ Hz, 2H), 6.49 (s, 2H), 2.73 (s, 3H). LC-MS (ESI, Positive): m/z 265 $[M+H]^+$. Anal. calcd. for $C_{16}H_{13}BrN_2O_2$: C, 55.67; H, 3.80; N, 8.12; %. Found: C, 55.55; H, 3.77; N, 8.07 %.

4-Acetyl-1-(2-oxo-2-*p*-tolylethyl)pyridiniumbromide (1f): Yellow solid. Yield 98 %. 1H NMR (400 MHz, $DMSO-d_6$): δ 9.22 (d, $J = 7.2$ Hz, 2H), 8.63 (d, $J = 7.0$ Hz, 2H), 7.98 (d, $J = 7.2$ Hz, 2H), 7.49 (d, $J = 6.9$ Hz, 2H), 6.56 (s, 2H), 2.79 (s, 3H), 2.46 (s, 3H). LC-MS (ESI, Positive): m/z 254 $[M+H]^+$. Anal. calcd. for $C_{16}H_{16}BrNO_2$: C, 57.50; H, 4.83; N, 4.19 %. Found: C, 57.30; H, 4.81; N, 4.15 %.

General procedure for the synthesis of ethyl 7-acetyl-3-(4-substituted benzoyl)-2-substituted indolizine-1-carboxylate: To a solution of 4-acetyl-1-[2-(4-substituted phenyl)-2-oxoethyl]pyridinium bromide (0.0156 mol), in dry dimethyl formamide, substituted ethyl propiolate (0.0156 mol), K_2CO_3 (0.0156 mol, 2.15 g) was added and the reaction mixture was stirred at room temperature for 0.5 h. Completion of the reaction was monitored on TLC. After completion of the reaction, the solvent was evaporated under reduced pressure and diluted with ethyl acetate. Organic layer was washed with water, brine and dried with sodium sulphate. The crude compounds were purified by column chromatography using 30 % *n*-hexane in ethyl acetate to afford the title compound **2a-2r** and physical constants are tabulated in Table-1.

Ethyl 7-acetyl-3-benzoylindolizine-1-carboxylate (2a): Yellow fluffy crystalline; IR (KBr, ν_{max} , cm^{-1}): 1685, 1626, 1597, 1575. 1H NMR (400 MHz, $CDCl_3$): δ 9.92 (d, $J = 7.2$ Hz, 1H), 8.99 (s, 1H), 7.87-7.84 (m, 3H), 7.63-7.53 (m, 4H), 4.42 (q, $J = 7.2$ Hz, 2H), 2.73 (s, 3H), 1.43 (t, $J = 7.2$ Hz, 3H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 195.82, 185.94, 163.72, 139.31, 138.32, 134.24, 131.97, 129.06, 128.85, 128.74, 128.52, 123.82, 121.05, 112.49, 109.38, 60.49, 26.17, 14.48. LC-MS (ESI, Positive): m/z 336 $[M+H]^+$. Anal. calcd. for $C_{20}H_{17}NO_4$: C 71.63, H 5.11, N 4.18 %. Found: C 71.72, H 5.21, N 4.11 %.

Diethyl 7-acetyl-3-benzoylindolizine-1,2-dicarboxylate (2b): Yellow crystalline compound; IR (KBr, ν_{max} , cm^{-1}): 1650,

TABLE-1
PHYSICO-CHEMICAL CONSTANTS OF ETHYL 7-ACETYL-3-(4-SUBSTITUTED
BENZOYL)-2-SUBSTITUTED INDOLIZINE-1-CARBOXYLATE ANALOGUES (2a-r)

Compound	m.f.	m.w.	R ¹	R ²	Yield (%) ^{a,b}	m.p. (°C)	cLogP ^c
2a	C ₂₀ H ₁₇ NO ₄	335	H	H	78	118-119	1.5990
2b	C ₂₃ H ₂₁ NO ₆	407	H	COOC ₂ H ₅	73	144-145	2.1814
2c	C ₂₀ H ₁₆ NO ₄ F	353	F	H	75	155-156	2.8312
2d	C ₂₁ H ₁₈ NO ₄ F	367	F	CH ₃	70	121-122	4.4772
2e	C ₂₂ H ₂₀ NO ₄ F	381	F	C ₂ H ₅	68	102-103	5.0062
2f	C ₂₃ H ₂₀ NO ₆ F	425	F	COOC ₂ H ₅	71	169-170	3.4512
2g	C ₂₀ H ₁₆ NO ₄ Cl	369	Cl	H	79	161-162	3.4012
2h	C ₂₁ H ₁₈ NO ₄ Cl	383	Cl	CH ₃	73	133-134	5.0472
2i	C ₂₃ H ₂₀ NO ₄ Cl	441	Cl	COOC ₂ H ₅	75	188-189	4.0212
2j	C ₂₀ H ₁₆ NO ₄ Br	413	Br	H	70	154-155	3.5512
2k	C ₂₁ H ₁₈ NO ₄ Br	427	Br	CH ₃	65	142-143	5.1972
2l	C ₂₃ H ₂₀ NO ₆ Br	485	Br	COOC ₂ H ₅	71	185-186	4.1712
2m	C ₂₂ H ₁₈ N ₂ O ₄	374	CN	CH ₃	72	146-147	3.8200
2n	C ₂₃ H ₂₀ N ₂ O ₄	388	CN	C ₂ H ₅	71	116-117	4.3490
2o	C ₂₄ H ₂₀ N ₂ O ₆	432	CN	COOC ₂ H ₅	69	171-172	2.7843
2p	C ₂₁ H ₁₉ NO ₄	349	CH ₃	H	61	151-152	3.1840
2q	C ₂₂ H ₂₁ NO ₄	363	CH ₃	CH ₃	55	109-110	4.8300
2r	C ₂₄ H ₂₃ NO ₆	421	CH ₃	COOC ₂ H ₅	65	178-179	3.8061

^aAll of the products were characterized by spectral and physical data; ^bYields after purification by column chromatography; ^ccLogP was calculated using ChemBioDraw Ultra 13.0v.

1605, 1591, 1569. ¹H NMR (400 MHz, CDCl₃): δ 9.33 (d, *J* = 7.2 Hz, 1H), 8.83 (s, 1H), 7.70-7.64 (m, 4H), 7.54-7.50 (m, 2H), 4.31 (q, *J* = 7.2 Hz, 2H), 3.62 (q, *J* = 7.2 Hz, 2H), 2.70 (s, 3H), 1.29 (t, *J* = 7.2 Hz, 3H), 1.01 (t, *J* = 7.2 Hz, 3H). LC-MS (ESI, Positive): *m/z* 408 [M+H]⁺. Anal. calcd. for C₂₃H₂₁NO₆: C 67.80, H 5.20, N 3.44 %. Found: C 66.93, H 5.27, N 3.17 %.

Ethyl 7-acetyl-3-(4-fluorobenzoyl)indolizine-1-carboxylate (2c): Yellow solid; IR (KBr, *v*_{max}, cm⁻¹): 1675, 1625, 1586. ¹H NMR (400 MHz, CDCl₃): δ 9.86 (d, *J* = 7.2 Hz, 1H), 8.99 (s, 1H), 7.90-7.83 (m, 2H), 7.83 (s, 1H), 7.62 (d, *J* = 7.2 Hz, 1H), 7.23 (t, *J* = 8.4 Hz, 2H), 4.42 (q, *J* = 7.2 Hz, 2H), 2.73 (s, 3H), 1.44 (t, *J* = 7.2 Hz, 3H). LC-MS (ESI, Positive): *m/z* 354 [M+H]⁺. Anal. calcd. for C₂₀H₁₆FNO₄: C 67.98, H 4.56, N 3.96 %. Found: C 67.81, H 4.31, N 3.71 %.

Ethyl 7-acetyl-3-(4-fluorobenzoyl)-2-methylindolizine-1-carboxylate (2d): Yellow crystalline compound; IR (KBr, *v*_{max}, cm⁻¹): 1665, 1635, 1575. ¹H NMR (400 MHz, CDCl₃): δ 9.01 (d, *J* = 8 Hz, 1H), 8.89 (s, 1H), 7.80-7.74 (m, 2H), 7.40 (d, *J* = 7 Hz, 1H), 7.18 (t, *J* = 8.4 Hz, 2H), 4.45 (q, *J* = 7.2 Hz, 2H), 2.69 (s, 3H), 2.24 (s, 3H), 1.45 (t, *J* = 7.2 Hz, 3H). LC-MS (ESI, Positive): *m/z* 368 [M+H]⁺. Anal. calcd. for C₂₁H₁₈FNO₄: C 68.66, H 4.94, N 3.81 %. Found: C 68.46, H 4.74, N 3.68 %.

Ethyl 7-acetyl-2-ethyl-3-(4-fluorobenzoyl)indolizine-1-carboxylate (2e): Dark yellow solid; IR (KBr, *v*_{max}, cm⁻¹): 1695, 1645, 1600. ¹H NMR (400 MHz, CDCl₃): δ 9.01 (d, *J* = 8 Hz, 1H), 8.90 (s, 1H), 7.79-7.76 (m, 2H), 7.42 (d, *J* = 7 Hz, 1H), 7.18 (t, *J* = 8.4 Hz, 2H), 4.45 (q, *J* = 7.2 Hz, 2H), 2.78 (q, *J* = 7.2 Hz, 2H), 2.69 (s, 3H), 1.45 (t, *J* = 7.2 Hz, 3H), 1.04 (t, *J* = 7.2 Hz, 3H). LC-MS (ESI, Positive): *m/z* 382 [M+H]⁺. Anal. calcd. for C₂₂H₂₀FNO₄: C 69.28, H 5.29, N 3.67 %. Found: C 68.92, H 5.12, N 3.51 %.

Diethyl 7-acetyl-3-(4-fluorobenzoyl)indolizine-1,2-dicarboxylate (2f): Yellow solid; IR (KBr, *v*_{max}, cm⁻¹): 1732, 1708, 1681, 1616, 1596. ¹H NMR (400 MHz, CDCl₃): δ 9.41 (d, *J* = 7 Hz, 1H), 8.99 (s, 1H), 7.79-7.76 (m, 2H), 7.60 (d, *J* =

7.2 Hz, 1H), 7.15 (t, *J* = 8.4 Hz, 2H), 4.39 (q, *J* = 7.2 Hz, 2H), 3.78 (q, *J* = 7.2 Hz, 2H), 2.71 (s, 3H), 1.37 (t, *J* = 7.2 Hz, 3H), 1.09 (t, *J* = 7.2 Hz, 3H). LC-MS (ESI, Positive): *m/z* 426 [M+H]⁺. Anal. calcd. for C₂₃H₂₀FNO₆: C 64.94, H 4.74, N 3.29 %. Found: C 64.78, H 4.69, N 3.19 %.

Ethyl 7-acetyl-3-(4-chlorobenzoyl)indolizine-1-carboxylate (2g): Light green solid; (KBr, *v*_{max}, cm⁻¹): 1695, 1680, 1618, 1591. ¹H NMR (400 MHz, CDCl₃): δ 9.86 (d, *J* = 7.2 Hz, 1H), 8.98 (s, 1H), 7.79 (d, *J* = 8.4 Hz, 2H), 7.65 (s, 1H), 7.61 (d, *J* = 7.2 Hz, 1H), 7.51 (d, *J* = 8.4 Hz, 2H), 4.41 (q, *J* = 7.2 Hz, 2H), 2.72 (s, 3H), 1.43 (t, *J* = 7.2 Hz, 3H). LC-MS (ESI, Positive): *m/z* 370 [M+H]⁺. Anal. calcd. for C₂₀H₁₆ClNO₄: C 64.96, H 4.36, N 3.79 %. Found: C 64.82, H 4.01, N 3.69 %.

Ethyl 7-acetyl-3-(4-chlorobenzoyl)-2-methylindolizine-1-carboxylate (2h): Light yellow crystalline compound; IR (KBr, *v*_{max}, cm⁻¹): 1681, 1608, 1589. ¹H NMR (400 MHz, CDCl₃): δ 9.98 (d, *J* = 7.2 Hz, 1H), 8.99 (s, 1H), 7.57 (d, *J* = 6.4 Hz, 1H), 7.50-7.37 (m, 4H), 4.42 (q, *J* = 7.2 Hz, 2H), 2.71 (s, 3H), 2.15 (s, 3H), 1.45 (t, *J* = 7.2 Hz, 3H). ¹³C NMR (400 MHz, CDCl₃): δ 195.87, 185.08, 164.46, 140.55, 140.20, 138.55, 134.47, 131.24, 130.92, 130.28, 128.50, 128.41, 127.44, 123.80, 120.74, 112.26, 109.18, 60.29, 26.17, 14.41, 12.84. LC-MS (ESI, Positive): *m/z* 384 [M+H]⁺. Anal. calcd. for C₂₁H₁₈ClNO₄: C 65.71, H 4.73, N 3.65 %. Found: C 65.46, H 4.81, N 3.47 %.

Diethyl 7-acetyl-3-(4-chlorobenzoyl)indolizine-1,2-dicarboxylate (2i): Yellow crystalline compound; IR (KBr, *v*_{max}, cm⁻¹): 1708, 1682, 1614, 1590. ¹H NMR (400 MHz, CDCl₃): δ 9.45 (d, *J* = 7.6 Hz, 1H), 8.98 (s, 1H), 7.67 (d, *J* = 8.4 Hz, 2H), 7.60 (d, *J* = 7.2 Hz, 1H), 7.44 (d, *J* = 8.4 Hz, 2H), 4.37 (q, *J* = 7.2 Hz, 2H), 3.75 (q, *J* = 7.2 Hz, 2H), 2.71 (s, 3H), 1.37 (t, *J* = 7.2 Hz, 3H), 1.09 (t, *J* = 7.2 Hz, 3H). LC-MS (ESI, Positive): *m/z* 442 [M+H]⁺. Anal. calcd. for C₂₃H₂₀ClNO₆: C 62.52, H 4.56, N 3.17 %. Found: C 62.69, H 4.39, N 3.01 %.

Ethyl 7-acetyl-3-(4-bromobenzoyl)indolizine-1-carboxylate (2j): Yellow solid; IR (KBr, *v*_{max}, cm⁻¹): 1701, 1687,

1622, 1586. ^1H NMR (400 MHz, CDCl_3): δ 9.87 (d, J = 8 Hz, 1H), 8.98 (s, 1H), 7.81 (s, 1H), 7.73-7.68 (m, 4H), 7.62 (d, J = 6.2 Hz, 1H), 4.42 (q, J = 7.2 Hz, 2H), 2.73 (s, 3H), 1.44 (t, J = 7.2 Hz, 3H). ^{13}C NMR (400 MHz, CDCl_3): δ 195.72, 184.58, 163.58, 138.50, 138.03, 134.43, 131.82, 130.56, 128.72, 128.62, 126.86, 123.43, 121.02, 112.67, 109.55, 60.56, 26.17, 14.48. LC-MS (ESI, Positive): m/z 414 $[\text{M}+\text{H}]^+$. Anal. calcd. for $\text{C}_{20}\text{H}_{16}\text{BrNO}_4$: C 57.99, H 3.89, N 3.38 %. Found: C 57.92, H 3.92, N 3.31 %.

Ethyl 7-acetyl-3-(4-bromobenzoyl)-2-methylindolizine-1-carboxylate (2k): Yellow solid; IR (KBr, ν_{max} , cm^{-1}): 1681, 1606, 1583. ^1H NMR (400 MHz, CDCl_3): δ 9.30 (d, J = 7 Hz, 1H), 8.97 (s, 1H), 7.65 (d, J = 10.4 Hz, 2H), 7.60 (d, J = 9 Hz, 2H), 7.47 (d, J = 7.2 Hz, 1H), 4.44 (q, J = 7.2 Hz, 2H), 2.70 (s, 3H), 2.28 (s, 3H), 1.46 (t, J = 7.2 Hz, 3H). ^{13}C NMR (400 MHz, CDCl_3): δ 195.89, 186.78, 164.54, 139.00, 137.84, 137.41, 133.69, 132.02, 130.60, 127.53, 127.34, 123.85, 121.12, 111.62, 108.55, 60.24, 26.09, 15.00, 14.45. LC-MS (ESI, Positive): m/z 428 $[\text{M}+\text{H}]^+$. Anal. calcd. for $\text{C}_{21}\text{H}_{18}\text{BrNO}_4$: C 58.89, H 4.24, N 3.27 %. Found: C 58.91, H 4.01, N 3.09 %.

Diethyl 7-acetyl-3-(4-bromobenzoyl)indolizine-1,2-dicarboxylate (2l): Dark brown crystalline compound; IR (KBr, ν_{max} , cm^{-1}): 1710, 1672, 1630, 1585. ^1H NMR (400 MHz, CDCl_3): δ 9.31 (d, J = 7 Hz, 1H), 8.80 (s, 1H), 7.72 (d, J = 8.4 Hz, 2H), 7.64 (d, J = 4 Hz, 1H), 7.61 (d, J = 8.4 Hz, 2H), 4.29 (q, J = 7.2 Hz, 2H), 3.65 (q, J = 7.2 Hz, 2H), 2.68 (s, 3H), 1.27 (t, J = 7.2 Hz, 3H), 1.03 (t, J = 7.2 Hz, 3H). LC-MS (ESI, Positive): m/z 486 $[\text{M}+\text{H}]^+$. Anal. calcd. for $\text{C}_{23}\text{H}_{20}\text{BrNO}_6$: C 56.80, H 4.15, N 2.88 %. Found: C 56.87, H 4.01, N 2.79 %.

Ethyl 7-acetyl-3-(4-cyanobenzoyl)-2-methylindolizine-1-carboxylate (2m): Yellow solid; IR (KBr, ν_{max} , cm^{-1}): 2231, 1707, 1684, 1624. ^1H NMR (400 MHz, CDCl_3): δ 9.47 (d, J = 7.2 Hz, 1H), 8.98 (s, 1H), 7.83-7.78 (m, 4H), 7.52 (d, J = 7.2 Hz, 1H), 4.45 (q, J = 7.2 Hz, 2H), 2.71 (s, 3H), 2.22 (s, 3H), 1.46 (t, J = 7.2 Hz, 3H). LC-MS (ESI, Positive): m/z 375 $[\text{M}+\text{H}]^+$. Anal. calcd. for $\text{C}_{22}\text{H}_{18}\text{N}_2\text{O}_4$: C 70.58, H 4.85, N 7.48 %. Found: C 70.39, H 4.91, N 7.69 %.

Ethyl 7-acetyl-3-(4-cyanobenzoyl)-2-ethylindolizine-1-carboxylate (2n): Light yellow solid; IR (KBr, ν_{max} , cm^{-1}): 2235, 1725, 1675, 1630. ^1H NMR (400 MHz, CDCl_3): δ 9.02 (d, J = 7 Hz, 1H), 8.94 (s, 1H), 7.81-7.55 (m, 5H), 7.42 (d, J = 7.2 Hz, 1H), 4.39 (q, J = 7.2 Hz, 2H), 2.75 (q, J = 7.2 Hz, 2H), 2.63 (s, 3H), 1.43 (t, J = 7.2 Hz, 3H), 1.03 (t, J = 7.2 Hz, 3H). LC-MS (ESI, Positive): m/z 389 $[\text{M}+\text{H}]^+$. Anal. calcd. for $\text{C}_{23}\text{H}_{20}\text{N}_2\text{O}_4$: C 71.12, H 5.19, N 7.21 %. Found: C 70.92, H 5.22, N 7.04 %.

Diethyl 7-acetyl-3-(4-cyanobenzoyl)indolizine-1,2-dicarboxylate (2o): Yellow solid; IR (KBr, ν_{max} , cm^{-1}): 2227, 1732, 1688, 1620, 1599. ^1H NMR (400 MHz, CDCl_3): δ 9.50 (d, J = 7.2 Hz, 1H), 9.01 (s, 1H), 7.80-7.74 (m, 4H), 7.59 (d, J = 7.2 Hz, 1H), 4.39 (q, J = 7.2 Hz, 2H), 3.78 (q, J = 7.2 Hz, 2H), 2.70 (s, 3H), 1.36 (t, J = 7.2 Hz, 3H), 1.08 (t, J = 7.2 Hz, 3H). LC-MS (ESI, Positive): m/z $[\text{M}+\text{H}]^+$: 433. Anal. calcd. for $\text{C}_{24}\text{H}_{20}\text{N}_2\text{O}_6$: C 66.66, H 4.66, N 6.48 %. Found: C 66.79, H 4.79, N, 6.51 %.

Ethyl 7-acetyl-3-(4-methylbenzoyl)indolizine-1-carboxylate (2p): Yellow solid; IR (KBr, ν_{max} , cm^{-1}): 1700, 1690, 1615. ^1H NMR (400 MHz, CDCl_3): δ 9.86 (d, J = 7.2

Hz, 1H), 8.97 (s, 1H), 7.85 (s, 1H), 7.75 (d, J = 8 Hz, 2H), 7.58 (d, J = 7.2 Hz, 1H), 7.33 (d, J = 7.2 Hz, 2H), 4.41 (q, J = 7.2 Hz, 2H), 2.71 (s, 3H), 2.47 (s, 3H), 1.42 (t, J = 7.2 Hz, 3H). LC-MS (ESI, Positive): m/z 350 $[\text{M}+\text{H}]^+$. Anal. calcd. for $\text{C}_{21}\text{H}_{19}\text{NO}_4$: C 72.19, H 5.48, N 4.01 %. Found: C 71.98, H 5.41, N 4.11 %.

Ethyl 7-acetyl-2-methyl-3-(4-methylbenzoyl)indolizine-1-carboxylate (2q): Yellow crystalline compound; IR (KBr, ν_{max} , cm^{-1}): 1697, 1683, 1625. ^1H NMR (400 MHz, CDCl_3): δ 9.19 (d, J = 7.2 Hz, 1H), 8.95 (s, 1H), 7.64 (d, J = 8.4 Hz, 2H), 7.43 (d, J = 7.2 Hz, 1H), 7.30 (d, J = 8.4 Hz, 2H), 4.43 (q, J = 7.2 Hz, 2H), 2.69 (s, 3H), 2.46 (s, 3H), 2.29 (s, 3H), 1.46 (t, J = 7.2 Hz, 3H). LC-MS (ESI, Positive): m/z 364 $[\text{M}+\text{H}]^+$. Anal. calcd. for $\text{C}_{22}\text{H}_{21}\text{NO}_4$: C 72.71, H 5.82, N 3.85 %. Found: C 72.63, H 5.88, N 3.76 %.

Diethyl 7-acetyl-3-(4-methylbenzoyl)indolizine-1,2-dicarboxylate (2r): Yellow crystalline compound; IR (KBr, ν_{max} , cm^{-1}): 1705, 1685, 1625, 1610. ^1H NMR (400 MHz, CDCl_3): δ 9.37 (d, J = 7.2 Hz, 1H), 8.97 (s, 1H), 7.65 (d, J = 8.0 Hz, 2H), 7.57 (d, J = 7.2 Hz, 1H), 7.26 (d, J = 8.4 Hz, 2H), 4.38 (q, J = 7.2 Hz, 2H), 3.72 (q, J = 7.2 Hz, 2H), 2.71 (s, 3H), 2.43 (s, 3H), 1.37 (t, J = 7.2 Hz, 3H), 1.05 (t, J = 7.2 Hz, 3H). LC-MS (ESI, Positive): m/z 422 $[\text{M}+\text{H}]^+$. Anal. calcd. for $\text{C}_{24}\text{H}_{23}\text{NO}_6$: C 68.40, H 5.50, N 3.32 %. Found: C 68.29, H 5.42, N 3.49 %.

Anticancer activity: Selected test compounds **2b**, **2h**, **2i**, **2q** and **2r** have been screened for *in vitro* anticancer activity against human cervix cancer cell line SiHa at 10, 20, 40 and 80 $\mu\text{g/mL}$ concentration using sulforhodamine B assay according to reported literature [47,48]. The results are tabulated in Table-2.

TABLE-2
in vitro ANTICANCER ACTIVITY OF TEST
COMPOUNDS **2b**, **2h**, **2i**, **2q** AND **2r** AGAINST
HUMAN CERVIX CANCER CELL LINE SiHa

Compound	Cell growth inhibition (%) ^a at concentration ($\mu\text{g/mL}$)			
	10	20	40	80
2b	91.0	-42.5	-53.9	-63.1
2h	100.0	97.1	86.9	81.9
2i	100.0	100.0	96.1	94.2
2q	100.0	100.0	-9.4	-22.4
2r	100.0	-30.9	-41.4	-54.8
ADR	-67.4	-70.0	-73.1	-74.1

^aAverage values of tested compounds with the reference adriamycin (ADR) as a reference positive control drug.

RESULTS AND DISCUSSION

The general route to obtain the title compounds **2a-r** is illustrated in **Scheme-I**. Compounds **1a-f** as intermediates were prepared by stirring 4-acetylpyridine with substituted phenacyl bromides separately in the presence of acetone medium at room temperature as shown in **Scheme-I**. The completion of reaction was monitored on thin layer chromatography (TLC), the solid deposited was filtered, dried under vacuum and recrystallized using ethanol as solvent. The yields of **1a-f** obtained were up to 98-99 % and characterization was achieved by proton NMR, LC-MS and elemental analysis.

Substituted indolizine compounds **2a-r** have been synthesized by the reaction between 4-acetyl-1-[2-(substituted phenyl)-2-oxoethyl]pyridin-1-ium bromide and substituted alkynes in presence of anhydrous potassium carbonate in dimethyl formamide medium as depicted in **Scheme-I**. The reaction completion was observed on TLC and all the products have been achieved within 0.5 h with constant stirring. Column chromatography was used to purify products using 60-120 mesh silica gel using 30 % *n*-hexane in ethyl acetate as a solvent and the yield was found to be 54-79 %. The title compounds have been characterized by IR, NMR, LC-MS and elemental analysis. *c*LogP of the compounds was calculated using ChemBioDraw Ultra 13.0v and found to be in the range of 1.5990-5.7262. IR (KBr) spectrum of the compounds **2a-r** had broad carbonyl (C=O) in the range of 1735-1650 cm⁻¹. Compounds **2m-2o** having aryl nitrile group (Ar-C≡N) exhibited absorbance at 2227-2231 cm⁻¹. The proton NMR spectrum exhibited quartet (-CH₂-) and triplet (-CH₃) in the range of 4.29-4.45 and 1.27-1.75 ppm, respectively for ethyl ester group (-COOC₂H₅) and singlet (-CH₃) in the range of 2.63-2.74 ppm for acetyl group (-COCH₃). ¹³C NMR spectrum of compound **2a** exhibited carbonyl carbon of acetyl group (CH₃CO) at 195.82 ppm. [M+H]⁺ peak for all the synthesized compounds is observed in the mass spectrum. Results of elemental analysis were in good agreement with the calculated values of the proposed title compounds **2a-r**.

Anticancer activity: Five of the selected test compounds **2b, 2h, 2i, 2q** and **2r** have been screened for *in vitro* anticancer activity against human cervix cancer cell line SiHa at 10, 20, 40 and 80 µg/mL concentration using sulforhodamine B assay [47,48]. The activity was carried out at Advanced Centre for Treatment, Research and Education in Cancer, (ACTREC) Mumbai using adriamycin (ADR) (doxorubicin) as positive control and the results are presented in Table-2. Three of the compounds such as **2b, 2q** and **2r** at 80 µg/mL exhibited remarkable lowest cell growth promotion against human cervix cancer cell line SiHa of -63.1, 22.4 and -54.8, respectively, when compared to standard adriamycin -74.1. *c*LogP of test compounds **2b, 2q** and **2r** was 2.1814, 4.83 and 3.8061, respectively.

Conclusion

The research work is focused on the efficient synthesis of substituted indolizines analogues and the reactions performed are eco-friendly. Yield of the products including intermediates were satisfactory. In addition, some of the selected test compounds are subjected for anticancer activity and compounds **2b, 2q** and **2r** were found to show dose dependent anticancer activity at 10, 20, 40 and 80 µg/mL.

ACKNOWLEDGEMENTS

The authors are grateful to Principal, Sahyadri Science College, Shimoga, India and Durban University of Technology, Durban, South Africa for facilities and encouragement. The authors also acknowledge to TATA Memorial Centre, Advanced Centre for Treatment Research and Education in Cancer, Mumbai, India for carrying out anticancer activities.

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