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

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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], (-)-monomorine [15], (-)-gephyrotoxin [16], etc. On the other hand, synthetic indolizine derivatives have been reported as calcium channel blockers [17], phospholipase A2 inhibitors [18], histamine H2-receptors antagonist [19] and 5-HT<sub>3</sub>-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 framework [28-32].

Typical molecular constructions of indolizines fall into three classes. (a) Condensation reactions of a 2-alkylpyrididine with acid anhydrides (Scholta reaction) [33] or α-haloketones (Tschitschibabin 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-methylyides generated from pyridinium salts under K\textsubscript{2}CO\textsubscript{3} [38,39], pyridine and carbones [40] or N-trimethylsilylmethylypyridinium triflates under fluoride ion [41] with acetylones or reaction of pyridinium N-methylyides 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 (1a-f, Scheme-1) for in vitro anticancer properties.

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 13C) were recorded at ambient temperature using CDCl\textsubscript{3}, DMSO-

General procedure for the synthesis of ethyl 7-acetyl-3-(4-substituted benzoyl)-2-substituted indolizine-1-carboxylate: To a stirred solution of 4-acetylpyridine (0.1 mol, 12.1 g) in dry acetone, 5 h, stir; (i) K\textsubscript{2}CO\textsubscript{3}, DMF, 0.5 h stir at room temperature

4-Acetyl-1-(2-oxo-2-phenylethyl)pyridinium bromide (1a): White solid. Yield 99 %. 1\textsuperscript{H} NMR (400 MHz, DMSO-

4-Acetyl-1-(2-oxo-2-tolylethyl)pyridinium bromide (1b): White solid. Yield 99 %. 1\textsuperscript{H} NMR (400 MHz, DMSO-

4-Acetyl-1-[2-(4-fluorophenyl)-2-oxoethyl]pyridinium bromide (1b): White solid. Yield 98 %. 1\textsuperscript{H} NMR (400 MHz, DMSO-

4-Acetyl-1-[2-(4-chlorophenyl)-2-oxoethyl]pyridinium bromide (1c): Light yellow solid. Yield 98 %. 1\textsuperscript{H} NMR (400 MHz, DMSO-

4-Acetyl-1-[2-(4-bromophenyl)-2-oxoethyl]pyridinium bromide (1d): Light yellow solid. Yield 99 %. 1\textsuperscript{H} NMR (400 MHz, DMSO-

4-Acetyl-1-[2-(4-cyanophenyl)-2-oxoethyl]pyridinium bromide (1e): Light brown solid. Yield 99 %. 1\textsuperscript{H} NMR (400 MHz, DMSO-

4-Acetyl-1-[2-(4-bromo-2-tolyethyl)pyridinium bromide (1f): Yellow solid. Yield 98 %. 1\textsuperscript{H} NMR (400 MHz, DMSO-

Ethyl 7-acetyl-3-benzoylindolizine-1-carboxylate (2a): Yellow fluffy crystalline; IR (KBr, \nu\textsubscript{max}, cm\textsuperscript{-1}): 1650, 1597, 1575. 1\textsuperscript{H} NMR (400 MHz, CDCl\textsubscript{3}): \delta 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), 1.46 (s, 3H). LC-MS (ESI (Positve)): m/z 254 [M+H]\textsuperscript{+}. Anal. calcd. for C\textsubscript{15}H\textsubscript{16}BrNO\textsubscript{2}: C, 57.50; H, 4.83; N, 4.17 %. Found: C, 57.30; H, 4.81; N, 4.19 %.

Diethyl 7-acetyl-3-benzoylindolizine-1,2-dicarboxylate (2b): Yellow crystalline compound; IR (KBr, \nu\textsubscript{max}, cm\textsuperscript{-1}): 1650,
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 426 [M+H]+. Anal. calcld. for C₂₁H₁₈NO₄Cl: C 65.71, H 4.73, N 3.65 %. Found: C 65.46, H 4.81, N 3.47 %.

**Ethyl 7-acetyl-3-(4-fluorobenzoyl)indolizine-1-carboxylate (2e):** Yellow solid; IR (KBr, νₐs, cm⁻¹): 1681, 1608, 1589. 1H NMR (400 MHz, CDCl₃): δ 9.86 (d, J = 7.2 Hz, 1H), 8.98 (s, 1H), 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. calcld. for C₂₂H₂₀NO₄: C 68.92, H 5.20, N 3.44 %. Found: C 66.93, H 5.27, N 3.17 %.

**Ethyl 7-acetyl-3-(4-chlorobenzoyl)indolizine-1-carboxylate (2f):** Yellow crystalline compound; IR (KBr, νₐs, cm⁻¹): 1681, 1616, 1608, 1591. 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), 7.75 (t, J = 8.4 Hz, 2H), 3.9 (q, J = 7.2 Hz, 2H), 2.7 (s, 3H), 1.29 (t, J = 7.2 Hz, 3H), 1.01 (t, J = 7.2 Hz, 3H). LC-MS (ESI, Positive): m/z 370 [M+H]+. Anal. calcld. for C₂₂H₂₂ClNO₄: C 66.94, H 4.74, N 3.29 %. Found: C 64.78, H 4.69, N 3.19 %.

**Ethyl 7-acetyl-3-(4-bromobenzoyl)indolizine-1-carboxylate (2g):** Light green solid; (KBr, νₐs, cm⁻¹): 1695, 1680, 1618, 1591. 1H NMR (400 MHz, CDCl₃): δ 9.86 (d, J = 7.2 Hz, 1H), 8.98 (s, 1H), 7.83 (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), 1.29 (t, J = 7.2 Hz, 3H), 1.05 (t, J = 7.2 Hz, 3H). LC-MS (ESI, Positive): m/z 380 [M+H]+. Anal. calcld. for C₂₃H₂₁BrNO₄: C 66.96, H 4.36, N 3.79 %. Found: C 66.82, H 4.01, N 3.69 %.

**Ethyl 7-acetyl-3-(4-fluorobenzyl)-2-methylindolizine-1-carboxylate (2h):** Light yellow crystalline compound; IR (KBr, νₐs, cm⁻¹): 1681, 1608, 1591. 1H NMR (400 MHz, CDCl₃): δ 9.96 (d, J = 7.2 Hz, 1H), 8.83 (s, 1H), 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 358 [M+H]+. Anal. calcld. for C₂₂H₂₀NO₆: C 67.98, H 4.56, N 3.96 %. Found: C 67.81, H 4.31, N 3.71 %.

**Diethyl 7-acetyl-3-(4-fluorobenzoyl)indolizine-1,2-dicarboxylate (2i):** Yellow solid; IR (KBr, νₐs, 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. calcld. for C₂₁H₁₈NO₄Cl: C 66.94, H 4.74, N 3.29 %. Found: C 64.78, H 4.69, N 3.19 %.

**Diethyl 7-acetyl-3-(4-chlorobenzoyl)indolizine-1,2-dicarboxylate (2j):** Yellow crystalline compound; IR (KBr, νₐs, cm⁻¹): 1708, 1682, 1614, 1590. 1H NMR (400 MHz, CDCl₃): δ 9.5 (d, J = 7.6 Hz, 1H), 8.97 (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), 3.5 (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. calcld. for C₂₁H₁₈ClNO₄: C 65.52, H 4.56, N 3.17 %. Found: C 62.69, H 4.39, N 3.01 %.
1622, 1586. 1H NMR (400 MHz, CDCl3): 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). 13C NMR (400 MHz, CDCl3): δ 195.72, 184.58, 163.58, 138.50, 134.43, 131.82, 130.56, 128.72, 128.62, 123.86, 121.02, 116.67, 109.55, 60.56, 26.17, 14.48. LC-MS (ESI, Positive): m/z 414 [M+H]+. Anal. calcd. for C26H23NO6: C 68.40, H 5.50, N 3.32 %. Found: C 68.29, H 5.43, N 4.11 %.

Ethyl 7-acetyl-3-(4-bromobenzoyl)indolizine-1,2-dicarboxylate (2q): Yellow solid; IR (KBr, νmax, cm⁻¹): 1710, 1652, 1610. 1H NMR (400 MHz, CDCl3): δ 9.37 (d, J = 7.2 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). LC-MS (ESI, Positive): m/z 364 [M+H]+. Anal. calcd. for C23H19BrNO4: C 71.71, H 5.82, N 3.32 %. Found: C 72.63, H 5.88, N 3.76 %.

Ethyl 7-acetyl-3-(4-methylbenzoyl)indolizine-1,2-dicarboxylate (2r): Yellow solid; IR (KBr, νmax, cm⁻¹): 1700, 1675, 1622, 1615. 1H NMR (400 MHz, CDCl3): δ 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 342 [M+H]+. Anal. calcd. for C22H20N2O4: C 70.59, H 5.98, N 3.32 %. Found: C 70.59, H 5.98, N 3.76 %.

**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 μg/mL concentration using sulforhodamine B assay according to reported literature [47,48]. The results are tabulated in Table-2.
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-1. 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. cLogP 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-CN) 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 (-CH) 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 (CH₂=CO) 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, Mumbai, India for carrying out anticancer activities.

**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.

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