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Evaluation of anticancer activity of novel synthetic phenylindolizine derivatives

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Abstract

A series of novel (4-chlorophenyl)(6,7-diamino-1-phenylindolizin-3-yl)methanone derivatives (4a-e) were successfully synthesized by deploying various acetylenes in reaction with pyridinium salts (3a-e). The reaction underwent 1,3-dipolar cycloaddition reaction, to yield diversity of this series of compounds. Synthesized compounds were confirmed by FTIR, ^{1}H NMR and ^{13}C NMR. The anti-cancer activity was carried against human cervical cancer cell and Adriamycin was used as positive control, in which compound **4a** and **4c** have shown significant potential. The newly synthesised motifs upheld the biological potential for further exploration.

Keywords: Adriamycin, anti-cancer, 1,3-dipolar cycloaddition reaction, electron-deficient, Indolizine.

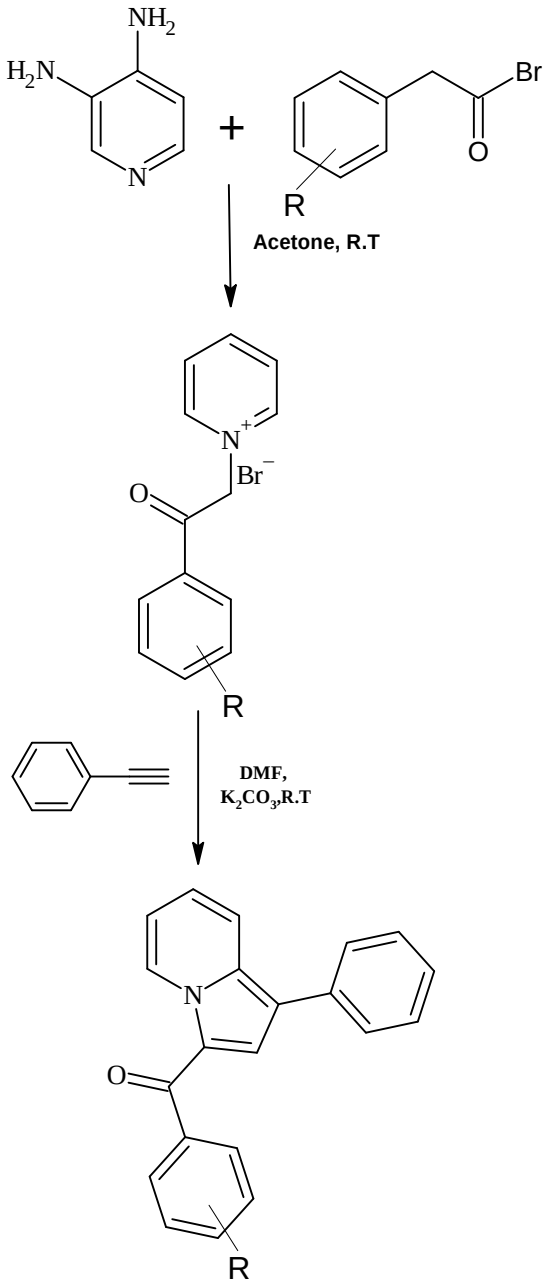
Introduction

Amid N-fused heterocyclic compounds, Indolizine, a prominent heterocyclic compound, serve as intriguing derivatives, prevailing in various pharmacological agents. This array of N-fused framework has been profusely found within the structure of therapeutic compounds and exhibits therapeutic effects. Including anti-cancer, antioxidant, anti-hypertensive, central nervous system, antibacterial and anti-HIV activities. The diverse pharmacological potential of heterocyclic compound bearing nitrogen containing heteroatom plays a vital role in medicinal drug, continuing intrigue with their synthesis and exploration. In accordance with these studies, in this article we report the synthesis and characterization of novel series of

indolizines i.e. a sub class of heteroaromatic compounds, containing two different fused rings as 5 membered and 6 membered rings with nitrogen as bridged atom. It is termed with several name in literature like pyridine, pyrrodine, pyrrocoline etc. The aromatized indolizine do not commonly appear in the environment, but reduced or substituted indolizines are usually encountered in nature. Several indolizine derivatives have been extracted from animals, insects, marine-lives, plants, swains nine, ipalbidine, tashirome, dehydrotylophorine, microbes and calprotectin. The significance of indolizines in drug design by medicinal chemists and challenge in developing indolizines with well-defined substitution patterns have made them appeal in target for both medicinal organic and synthetic chemists. The synthesis of indolizines derivatives have various new and attractive methodologies which have been invented and the synthesis of a wide range of compounds which have exhibited several biological activities. The indolizine syntheses have various routes which can be classified as cycloaddition reaction, Tschichibabin reaction and intramolecular cyclisation. Even though the various approaches initiate from the substrates like nitrogen in six membered ring like quinoline, pyridine and isoquinoline. Furthermore, new major indolizines act as sensors for modern technologies because of its capacity to form surface film.

Experimental Methods

The Synthesis of (4-chlorophenyl)(6,7-diamino-1-phenylindolizin-3-yl)methanone was carried via 1,3-dipolar cycloaddition reaction. Chemical were procured from commercial source such TCI chemicals, and spectrochem Ltd. All the melting points were determined in an open capillary and were uncorrected. IR spectra were recorded on Bruker alpha FT IR spectrophotometer, ^{1}H NMR spectra were measured on Bruker AV 400 MHZ using $CDCl_3$ as solvent. Chemical shifts are expressed in δ ppm. Mass spectra were performed on a Joel JMS-D 300 mass spectrometer. All the reactions were monitored by TLC and were observed under ultraviolet infrared spectra, further purification was done by column chromatography. All the reagents used were of AR grade and they were again purified by distillation.



R=Cl, OMe, F, CN, Br

General procedure for the preparation of 3, 4-diamino-1-(2-(4-bromophenyl)-2-oxethyl) pyridine-1-ium bromide 3a

To stirred a solution of 3,4-diamino pyridine (1g, 0.0092 mol) in acetone (15 mL), 4-chlorophenacylbromide (2.139g, 0.0092 mol) was added and stirred for 3-4 hours at room temperature. Salt formed was separated and dried under reduced pressure to obtained pure 4.8g (97.13%) of 3,4-diamino-1-(2-(4-bromophenyl)-2-oxethyl)pyridine-1-ium bromide **3a**. Similarly, other salts were prepared in the same protocol **(3b-e).**

Preparation of (4-chlorophenyl)(6,7-diamino-1-phenylindolizin-3-yl)methanone 4a

To stirred solution of 3,4-diamino-1-(2-(4-chlorophenyl)-2-oxethyl)pyridine-1-iumbromide **3a** (0.5g, 0.0015mol) in dimethyl formamide, was added phenyl acetylene(0.153g, 0.0015mol) and potassium carbonate(0.248g, 0.0018mol). The reaction was carried at room temperature for 45 minutes. The progress of reaction was monitored by thin layer chromatography and observed under ultraviolet light. After completion of the reaction, the crude product obtained was poured in crushed ice and further, it was diluted with ethyl acetate and collected in separating funnel followed by wash with distilled water, brine solution and dried over anhydrous sodium sulphate, the organic layer obtained was left over evaporation.

(4-Chlorophenyl)(6,7-diamino-1-phenylindolizin-3-yl)methanone 4a

IR (KBr, cm^{-1}); 3445.28(Ar-NH2), 1634(C=O), 935.93(Ar-C-Cl). **^{1}H NMR** ($CDCl_3$ 400 MHz); δ in ppm: 8.09-8.07 (1H, m), 7.87-7.81(1H, m), 7.62-7.60(1H, m), 7.39-7.37(1H, m), 7.25(1H, s), 7.19-7.15(1H, m), 6.79-6.77(1H, m). 4.07-4.13(1H, s). **^{13}C NMR** ($CDCl_3$, 150MHz); 145.81, 144.75, 133.74, 132.40, 128.98, 128.74, 127.33, 125.71, 125.02, 117.63, 112.81, 112.70, 108.29, 106.91, 77.46, 77.34, 77.14, 76.82, 75.57, 71.72

(6,7-Diamino-1-phenylindolizin-3-yl)(4-methoxyphenyl)methanone 4b

IR (KBr, cm^{-1}); 3464.02(Ar-NH2), 1632(C=O), 1295.85(Ar-$COCH_3$). **^{1}H NMR** ($CDCl_3$ 400 MHz); δ in ppm: 8.06-8.04(1H, m), 7.87-7.85(1H, m), 7.73-7.60(1H, d), 7.58(1H, s), 7.14-7.10(1H, m), 6.96-6.94(1H, m), 6.74-6.72(1H, m), 4.00 (1H, s). **^{13}C NMR** ($CDCl_3$ 150MHz); 167.03, 159.65, 145.80, 139.90, 136.14, 127.37, 125.55, 124.52, 117.36, 114.21, 112.31, 107.32, 77.47, 77.15, 76.83.

(4-Bromophenyl)(6,7-diamino-1-phenylindolizin-3-yl)methanone 4c

IR (KBr, cm^{-1}); 3252(Ar-NH2), 1710(C=O), 850(Ar-C-Br). **^{1}H NMR** ($CDCl_3$ 400 MHz); δ in ppm: 8.10-8.07(1H, m), 7.83-7.80(1H, m), 7.59-7.60(1H, m), 7.24-7.14(1H, m), 6.55-6.52(1H, m), 7.25(1H, s), 6.77-6.78(1H, d), 4.24(1H, s). **^{13}C NMR** ($CDCl_3$ 150MHz); 147.70, 145.84, 144.80, 132.85, 131.94, 127.64, 125.71, 125.05, 121.96, 117.69, 115.76, 112.73, 108.31, 77.44, 77.11, 76.80, 70.40.

4-(6,7-Diamino-1-phenylindolizine-3-carbonyl)benzonitrile 4d

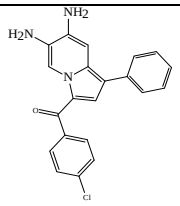
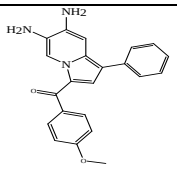
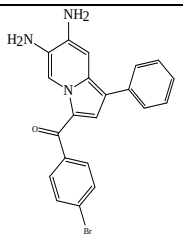
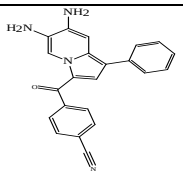
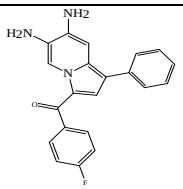
IR (KBr, cm^{-1}); 3358(Ar-NH_2), 1716(C=O), 2229(Ar-C-CN). **^{1}H NMR** ($CDCl_3$ 400 MHz); δ in ppm: 8.12-8.10(1H, m), 8.03-8.01(1H, m), 8.00-7.91(1H, m), 7.69-7.67(1H, m), 7.67-7.66(1H, m), 7.62-7.60(1H, m), 7.25(1H, s), 7.20-7.19(1H, m), 6.82-6.80(1H, m). **^{13}C NMR** ($CDCl_3$ 150MHz); 166.28, 165.90, 151.62, 146.01, 143.70, 138.36, 132.64, 126.41, 125.91, 125.61, 117.88, 113.15, 112.12, 111.08, 109.64, 109.34, 107.32, 77.49, 77.38, 77.17, 76.85, 77.65.

(6,7-Diamino-1-phenylindolizin-3-yl)(4-fluorophenyl)methanone 4e

IR (KBr, cm^{-1}); 3416(Ar-NH_2), 1758(C=O), 1391 (Ar-C-F). **^{1}H NMR** ($CDCl_3$ 400 MHz); δ in ppm: 8.11-8.10(1H, m), 7.93-7.90(1H, d), 7.89-7.80(1H, m), 7.63-7.61(1H, d), 7.25(1H, s), 7.18-7.11(1H, m), 7.09(1H, s), 6.95-6.93(1H, m), 6.78-6.77(1H, m),

4.23(1H, s). **^{13}C NMR** ($CDCl_3$ 150MHz); 163.97, 161.52, 145.74, 144.94, 130.06, 130.05, 130.04, 128.04, 127.77, 127.70, 125.66, 124.87, 117.49, 115.88, 115.64, 112.86, 112.55, 107.83, 77.50, 77.39, 77.18, 76.86.

Table:1 Physiochemical Characteristics of Indolizine derivatives **(4a-e)**

Code	Structure	Molecular formula	Molecular Weight	Melting Point in ^{0}C	Yield
4a		$C_{21}H_{16}ClN_3O$	361.82	110-111	92%;
4b		$C_{22}H_{19}N_3O_2$	3357.41	119-120	81%
4c		$C_{21}H_{16}BrN_3O$	406.28	112-113	90%
4d		$C_{22}H_{16}N_4O$	352.39	130-131	70%
4e		$C_{22}H_{18}N_2O_2$;	345.39	150-152	92%

Anti-cancer Activity

Screening methods of anticancer activity:

The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. For the present screening experiment, cells were inoculated into 96 well microtiter plates in 100 µL at plating densities as shown in the study details above, depending on the doubling time of individual cell lines. After cell inoculation, the microliter plates were incubated at 37° C, 5% CO2, 95% air and 100% relative humidity for 24 h before the addition of experimental drugs. Different extracts were initially solubilized in dimethyl sulfoxide at 100 mg/ml and diluted to 1mg/ml using water and stored frozen before use. At the time of drug addition, an aliquot of frozen concentrate (1 mg/ml) was thawed and diluted

to 100 µg/ml, 200 µg/ml, 400 µg/ml and 800 µg/ml with complete medium containing test article. Aliquots of 10 µl of these different drug dilutions were added to the appropriate microtiter wells already containing 90 µl of the medium, resulting in the required final drug concentrations, i.e. 10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml. After compound addition, plates were incubated at standard conditions for 48 h and assay was terminated by the addition of cold TCA. Cells were fixed in-situ by the gentle addition of 50 µl of cold 30 % (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 µl) at 0.4% (w/v) in 1% acetic acid was added to each of the wells, and plates were incubated for 20 min at room temperature. After staining, the unbound dye was recovered, and the residual dye was removed by washing five times with 1% acetic acid. The plates were air dried. The bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on a plate reader at a wavelength of 540 nm with 690 nm reference wavelength. Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent Growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control. All synthesized substituted indolizine derivatives as **4a, 4b, 4c, 4d** and **4e** were subjected for anti-cancer activity through human cervix cancer cell line SiHa and tested at concentrations 10µg/mL, 20µg/mL, µg/mL and 80 µg/mL. The compound **4a** and **4c** shown good anti-cancer properties against human cervix cancer cell line SiHa.

Table.2: Anti-cancer Activity against Human Cervic Cancer Cell Line SiHa

Cell growth inhibition (%), concentration (µg/mL)				
Compounds	**10**	**20**	**40**	**80**
4a	100	-31.21	-42.01	-56-6.31
4b	100	100	97.01	95.35
4c	100	-38.21	-40.61	-51.92
4d	100	100	-9.01	-20.81
4e	100	95.01	84.36	97.35
ADR	-67	-70	-73	-74

Results and Discussion

The present research article plays a vital role on the effective synthesis of substituted indolizine derivatives. We have successfully synthesized indolizine derivatives **4a-e** represented in the above Scheme. Some of the tested compounds have been screened for anticancer activity against SiHa cell lines. The compounds **4a** and **4c** exhibited significant anticancer activity.

Declaration of competing interest

None

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