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Graphical Abstract

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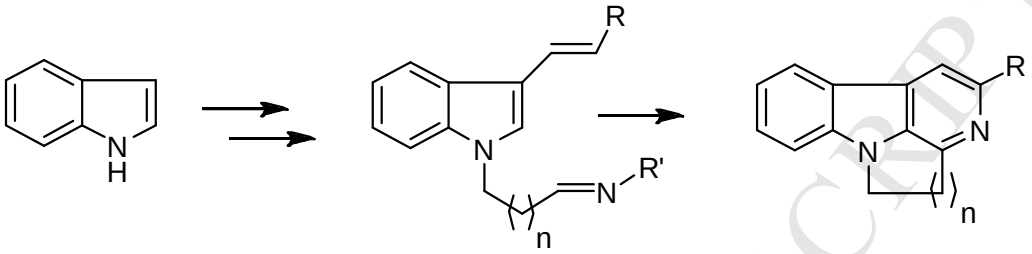

Synthesis of canthine analogues using intramolecular Aza-Diels-Alder strategy and evaluation of their activity against HeLa cervical cancer cells

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Abstract
Synthesis of six new canthine analogues has been developed using a one pot intramolecular Aza-Diels-Alder (ADA) reaction as the key step. The synthesized compounds demonstrated moderate activity against HeLa cervical cancer cell lines.

Key Words: intramolecular Aza-Diels-Alder reaction, canthine, hydrazone, HeLa cells

1. Introduction

Canthine alkaloids possess a tetracyclic skeleton having a β-carboline scaffold with an additional D ring. Some naturally occurring alkaloids possessing this skeleton are shown in **fig. 1.** There are several synthetic methods reported for these alkaloids because they show a wide range of pharmacological activities such as antiviral and antifungal properties as well as potent cytotoxicity against a variety of cell lines.[1] Although, Pictet-Spengler condensations and Bischler-Napieralski reactions[2] are common key steps for the synthesis of these compounds, there are also a few reports describing the use of ADA reactions.[3] The ADA strategy has also been used for the synthesis of other heterocyclic systems.[4]

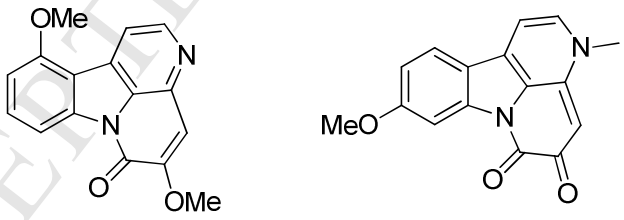

Fig. 1: Alkaloids possessing the canthine framework

In continuation with our interest in the synthesis of β-carboline systems,[5] and considering the medicinal importance of these scaffolds, we envisaged to apply the intramolecular ADA strategy for the synthesis of unnatural canthine alkaloids. The present work describes the synthesis of six new derivatives of canthine alkaloids and evaluation of their activity against HeLa cervical cancer cell lines.

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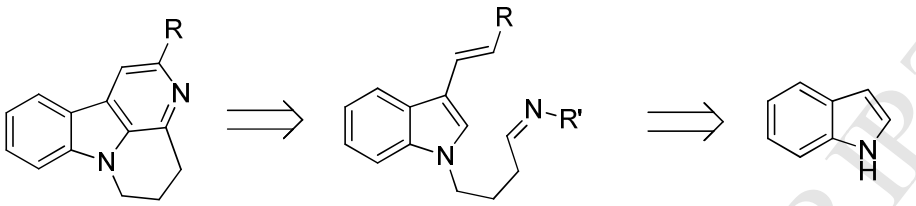
2. Results and Discussion

The synthesis was planned as shown in **scheme 1**, wherein simultaneous formation of rings C and D was envisioned.

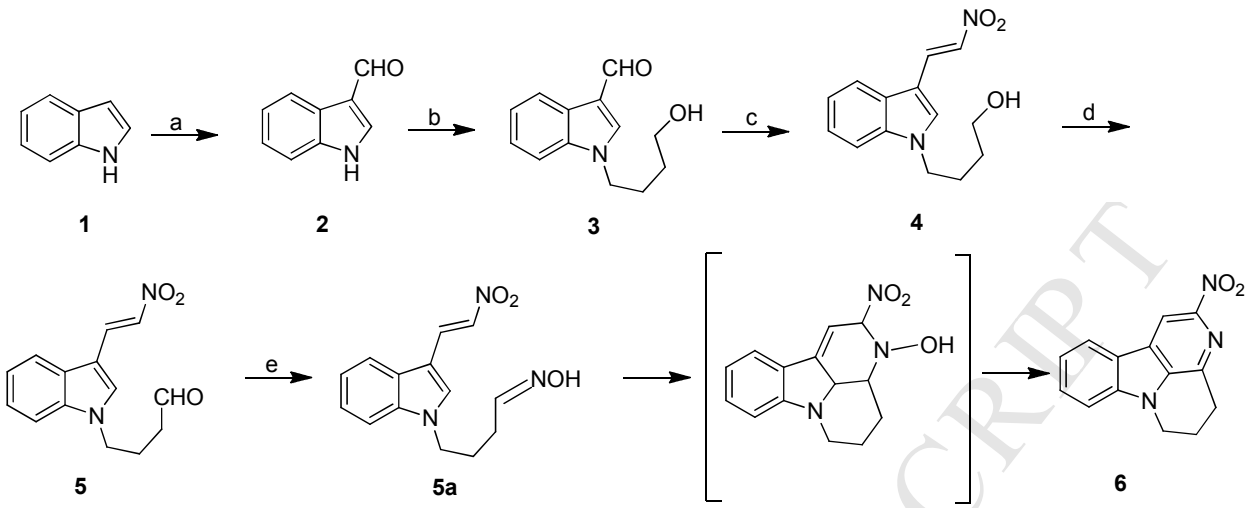


R= -NO_2, -COOEt, Ph, Me, R'= OH, NH_2

Scheme 1: Retrosynthetic analysis for canthine derivatives

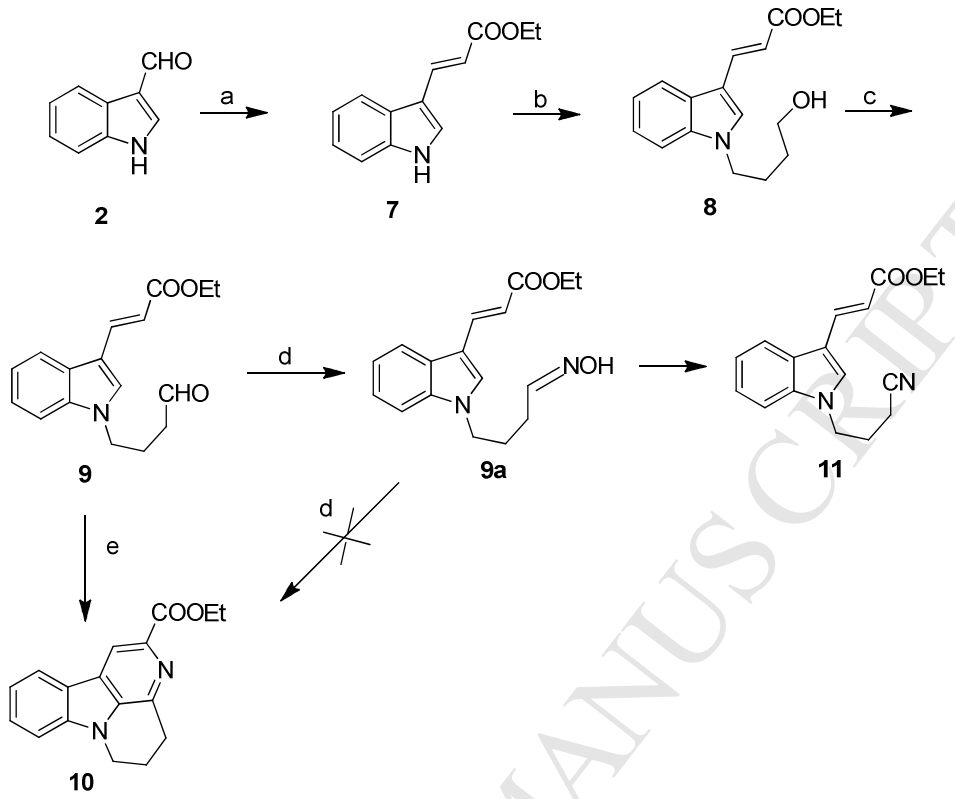
Accordingly, indole **1** was formylated using Vilsmeier-Haack reaction to give indole-3-carbaldehyde **2**, which was *N*-alkylated using 4-chlorobutan-1-ol affording compound **3** in 85% yield. Henry reaction using compound **3** furnished nitrostyrene **4** which on further oxidation using *o*-iodoxybenzoic acid (IBX) produced aldehyde **5**. Considering our earlier success in one pot electrocyclization reactions[5a,5b,6] a similar strategy was planned using intramolecular ADA reaction. The required aza dienophile was produced in situ by treatment of aldehyde **5** with hydroxylamine hydrochloride and sodium acetate in toluene. The reaction mixture was further refluxed for 72 hours, resulting in an oily product in 67% yield (**Scheme 2**).The spectral data was consistent with structure **6** for the product. The formation of this product could be explained by the intramolecular ADA reaction of the initially formed oxime followed by aromatization of the Diels-Alder cycloadduct. To confirm the steps in this one pot sequence, intermediate oxime **5a** was isolated and characterized by quenching the reaction after two hours of refluxing.

Thus, canthine analogue **6** was successfully synthesized using intramolecular ADA reaction in 45% overall yield.



Scheme 2: Reagents and Conditions: a) DMF, $POCl_3$, rt, 5 h, 95%; b) KOH, DMSO, 4-chlorobutan-1-ol, rt, 5 h, 85%; c) CH_3NO_2, NH_4OAc, AcOH, 80 °C, 3 h, 87%; d) IBX, EtOAc, 80 °C, 8 h, 95%; e) $NH_2OH.HCl$, NaOAc, toluene, 110 °C, 72 h, 67%.

With the aim to obtain the other derivative with R=COOEt, Wittig reaction of aldehyde **3** using carbethoxymethylenetriphenyl phosphorane was carried out to get olefin **8** in about 60% yield. Due to the poor yield in Wittig reaction, the reaction sequence was altered as shown in **scheme 3**. Thus, indole-3-carbaldehyde **2** was first treated with stable Wittig salt, to give the Wittig product **7**, which was then *N*-alkylated resulting in compound **8**. Further, oxidation of **8** to aldehyde **9** using IBX and then refluxing in toluene in presence of hydroxylamine hydrochloride and sodium acetate furnished a solid product. From the spectral data the structure was identified as nitrile **11** and not **10** as was expected. The possible reason for the formation of nitrile could be the rapid dehydration of intermediate oxime **9a.** The intermediate **9a** was isolated and characterized by quenching the reaction after one hour of refluxing.



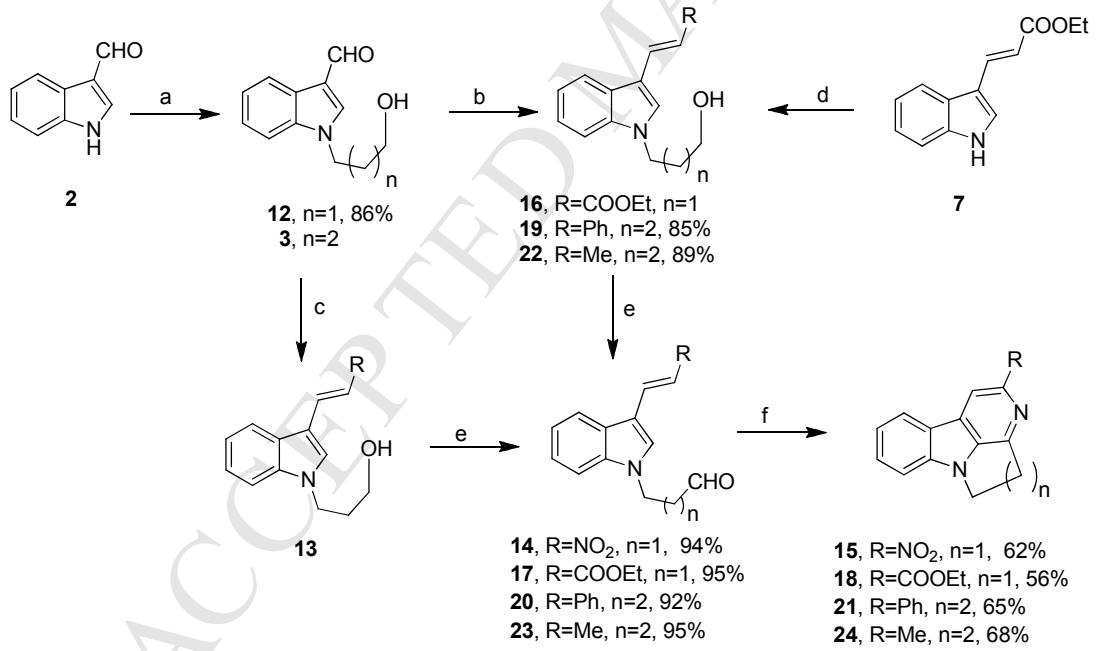
Scheme 3: Reagents and Conditions: a) $PPh_3CHCOOEt$, toluene, reflux, 6 h, 92%; b) KOH, DMSO, 4-chlorobutan-1-ol, rt, 7 h, 87%; c) IBX, EtOAc, 80 °C, 8 h, 96%; d) $NH_2OH.HCl$, NaOAc, toluene, 110 °C, 10 h, 90%; e) NH_2-$NH_2.H_2O$, toluene, reflux, 60 h, 65%.

There are a few reports[7] wherein –CN has been used as a dienophile although it requires harsh conditions. Thus, it was decided to make use of nitrile functionality as a hetero dienophile for the synthesis of the targeted ester derivative. Nitrile **11** was synthesized using an alternate short route of alkylation of Wittig product **7** using 4-bromobutyronitrile in good yield. Considering the high temperature requirement for the DA reactions using nitrile, compound **11** and indium chloride as an activating catalyst were adsorbed on silica gel and the solid mixture was heated on a sand bath. To our surprise, color of the reaction mixture changed at 180^{o}C and complete consumption of the starting material was seen on TLC plate. However, all the attempts to isolate and purify the product failed. The reaction of nitrile **11** was also tried under different conditions including heating in high boiling solvents like *o*-dichlorobenzene and sulfolane and even by using microwave conditions. But in these cases, starting material was recovered completely.

The above results compelled us to change the route. To achieve the synthesis of the target, it was envisaged to use hydrazone in the intramolecular ADA reaction. Literature survey revealed the use of *N*, *N*-dimethylhydrazone as a part of diene, however, to the best of our knowledge only a few reports described its use as a dienophile.[8] Use of unsubstituted hydrazone as an aza dienophile has not yet been explored. Thus, to investigate this possibility, aldehyde **9**

was refluxed with hydrazine hydrate in toluene, expecting in situ formation of hydrazone and further intramolecular ADA reaction (**Scheme 3**).The oily liquid obtained was shown to be compound **10** from the spectral data. Aromatization of the initially formed cycloadduct by the loss of $-NH_3$ in the same pot, explains the formation of product **10** in 50% overall yield. The aldehyde **5** was also treated with hydrazine hydrate and further refluxed to get compound **6** in 55% yield which was comparatively less than the earlier reaction using oxime **5a**.

Elated by the success in the use of hydrazone as a dienophile, we then planned for the synthesis of some more unnatural tetracyclic analogues. Thus, as shown in **scheme 4**, aldehyde **2** was *N*-alkylated using 3-chloropropan-1-ol to get **12** which during Henry reaction provided olefin **13**. Simultaneously, alkylation of Wittig product **7,** provided olefin **16** and Wittig reaction of **3** with benzylbromotriphenylphosphorane and ethyliodotriphenylphosphorane afforded olefins **19** and **22** respectively. Subsequent oxidation of **13**, **16**, **19** and **22** furnished aldehydes **14**, **17**, **20** and **23** respectively. The targeted canthine derivatives **15**, **18**, **21** and **24** were obtained by following a similar reaction sequence as before. Thus, treatment of the aldehydes with hydrazine hydrate and further heating the hydrazone formed in situ, produced the required unnatural canthine analogues. All the new compounds were characterized using spectral and analytical data.



Scheme 4: Reagents and Conditions: a) KOH, DMSO, 3-chloro-1-propanol, rt, 5 h; b)PPh_3CH_2RBr (R=Ph/Me), nBuLi, THF, 0 °C, 30 min; c) NO_2Me, NH_4OAc, AcOH, 80 °C, 3 h, 88%; d) KOH, DMSO, 3-chloro-1-propanol, rt, 7 h, 85%; e) IBX, EtOAc, 80 °C, 8 h; f) NH_2-$NH_2.H_2O$, toluene, reflux, 60 h.

2.1 Biological assay

After completing the synthesis of the unknown canthine analogues, biological evaluation of these compounds was undertaken for testing the activity against HeLa cervical cancer cell lines. As the activity of canthines was not studied against HeLa cell lines earlier, these cell lines were chosen. Thus the compounds **6**, **10**, **15** and **18** were dissolved in DMSO and diluted to micro molar (µM) concentration. The cell viability study was performed at an interval of 24 h and 48 h. At the end of 48 h, 59% cell viability was observed at µM concentration. The IC_{50} values are shown in **table 1**. The IC_{50} values indicate that the ester group shows preference over the nitro group. The ester group may help in interaction with the cellular surface which enhances drug uptake. Also, five membered D-ring is preferred over the six membered ring according to the IC_{50} values.

Table 1: IC_{50} values for canthine analogues

Compound No.	IC_{50}/µM
6	82.3±0.006
10	23.4±0.012
15	34.6±0.009
18	22.8±0.016

3. Conclusion

Six unnatural canthine alkaloids were synthesized using a new route wherein one pot intramolecular ADA reaction was the key step. Unprecedented use of unsubstituted hydrazone as an aza dienophile has been investigated. The canthine derivatives show moderate potential in inhibiting the HeLa cervical cancer cells at micro molar concentration with IC_{50} values in the range of 22-83µM.

4. Experimental

4.1 General Information

All reactions were carried out under an inert atmosphere with dry solvents, unless otherwise stated. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (Kieselgel 60 F254, Merck). Visualization of the spots on TLC plates was achieved either by UV light or by staining the plates in 2, 4-dinitrophenylhydrazine/anisaldehyde and charring on hot plate. All products were characterized by ^{1}H NMR and ^{13}C NMR, IR and HRMS/elemental analysis. ^{1}H NMR and ^{13}C NMR were recorded on Varian Mercury 300 MHz and 75 MHz

instrument respectively. Chemical shifts are expressed in parts per million values and ^{1}H NMR spectra are referenced to 0.00 ppm for Me_4Si (TMS) and ^{13}C NMR spectra are referenced to 77.00 ppm for $CDCl_3$. Peak multiplicities are designated by the following abbreviations: s, singlet; brs, broad singlet; d, doublet; t, triplet; q, quartet; quint, quintet; m, multiplet; exch, D_2O exchangeable; J, coupling constant in Hertz. IR spectra were obtained on a Nicolet 5700 and Bruker instrument with samples loaded as thin films on KBr plate, neat or with CH_2Cl_2 as indicated. HRMS spectra were obtained on a Micromass Q-TOF apparatus. Elemental analyses were recorded on Flash E. A. 1112 Thermo instrument. Melting points recorded are uncorrected. Column chromatography on silica gel (100-200 mesh) was performed with reagent grade ethyl acetate and hexane as an eluent.

4.2. 1H-Indole-3-carbaldehyde (**2**). Prepared according to literature procedure[9] (Mp 195 °C).

*4.3. General procedure for N-alkylindoles, **3**, **8**, **12**, **16***. To a solution of DMSO (10 ml) taken in a round bottom flask was added KOH (2 eq.) and the solution was stirred at room temperature for 15 min. The indole derivative **2** or **7** (1 eq.) was then added and the mixture was further stirred for 1 h, after which 4-chlorobutan-1-ol or 3-chloropropan-1-ol (1.5 eq.) was added drop wise to the reaction mixture. The reaction was monitored by TLC to completion within 5 h. The reaction mixture was then quenched by adding water and was extracted with ethyl acetate. Evaporation of the solvent under reduced pressure followed by column chromatography gave the required *N*-alkylated compounds, **3**/**8**/**12**/**16**.

4.3.1. 1-(4-Hydroxybutyl)-1H-indole-3-carbaldehyde (**3**). White solid (85%); mp 75-77 °C; FTIR (KBr cm^{-1}) 3389, 2862, 2361, 1623, 1524, 1388; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.85 (1H, s, -C*H*O), 8.24-8.28 (1H, m, Ar*H*), 7.68 (1H, s, Ar*H*), 7.25-7.37 (3H, m, Ar*H*), 4.16 (2H, t, *J*=6.2 Hz, -NCH_2), 3.63 (2H, t, *J*=7.2 Hz, -OCH_2), 2.77 (1H, brs, exch., -O*H*), 1.95 (2H, quint, *J*=7.7 Hz, -CH_2-), 1.55 (2H, quint, *J*=6.2 Hz, -CH_2-); ^{13}C NMR (75 MHz, $CDCl_3$) δ:184.6, 138.7, 137.0, 125.1, 123.8, 122.8, 121.8, 117.7, 110.1, 61.6, 46.9, 29.4, 26.2; Found: C 70.97, H 6.92, N 6.52; $C_{13}H_{15}NO_2$ requires: C 71.88, H 6.91, N 6.45 %.

4.3.2. (E)-Ethyl 3-(1-(4-hydroxybutyl)-1H-indol-3-yl)acrylate (**8**). Pale yellow solid (87%); mp 85-87 °C; FTIR (KBr cm^{-1}) 3378, 1713, 1613, 1527, 1163; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.83-7.90 (2H, m, Ar*H*), 7.33 (2H, d, *J*=6.6 Hz, Ar*H*, ArC*H*=CH-), 7.19-7.28 (2H, m, Ar*H*), 6.38 (1H, d, *J*=15.9 Hz, -C*H*COOEt), 4.24 (2H, q, *J*=7.1 Hz, -CH_2CH$_3$), 4.14 (2H, t, *J*=6.8 Hz, -NCH_2), 3.62 (2H, t, *J*=6.0 Hz, -OCH_2), 1.93 (2H, quint, *J*=7.1 Hz, -CH_2-), 1.73 (1H, brs, exch, -O*H*), 1.54 (2H, quint, *J*=7.1 Hz, -CH_2-), 1.33 (3H, t, *J*=7.1 Hz, -CH$_2$CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 168.2, 137.9, 137.1, 132.1, 126.0, 122.7, 121.1, 120.5, 112.3, 111.9, 110.0, 61.9, 60.0, 46.4, 29.7, 26.5, 14.5; HRMS (ESI) (M+H): 288.1607; Calculated for $C_{17}H_{22}NO_3$ 288.1599.

4.3.3. 1-(3-Hydroxypropyl)-1H-indole-3-carbaldehyde (**12**). Sticky solid (86%); FTIR (KBr cm^{-1}) 3382, 2869, 1641, 1526, 1390, 1168; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.86 (1H, s, -C*H*O), 8.25-8.28 (1H, m, Ar*H*), 7.76 (1H, s, Ar*H*), 7.37-7.43 (1H, m, Ar*H*), 7.27-7.34 (2H, m, Ar*H*), 4.33

(2H, t, *J*=7.2 Hz, -NC*H*$_2$), 3.61 (2H, t, *J*=5.8 Hz, -OC*H*$_2$), 3.07 (1H, brs, exch., -O*H*), 2.07 (2H, m, -C*H*$_2$-); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 184.8, 139.3, 137.1, 125.2, 123.9, 122.8, 121.8, 117.7, 110.1, 58.4, 43.5, 31.8; HRMS (ESI) (M+H) 204.1027; Calculated for $C_{12}H_{14}NO_2$ 204.1024.

4.3.4. (E)-Ethyl 3-(1-(3-hydroxypropyl)-1H-indol-3-yl)acrylate (**16**). Sticky solid (0.97 g, 85%); FTIR (KBr cm^{-1}) 3375, 1691, 1618, 1163; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.84-7.92 (2H, m, Ar*H*, ArC*H*=CH-), 7.22-7.36 (4H, m, Ar*H*), 6.42 (1H, d, *J*=15.9 Hz, -C*H*COOEt), 4.22-4.29 (4H, m, -NC*H*$_2$, -C*H*$_2$CH$_3$), 2.14-2.27 (4H, m, -OC*H*$_2$, -C*H*$_2$-), 1.88 (1H, brs, exch, -O*H*), 1.34 (3H, t, *J*=7.1 Hz, -CH$_2$C*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 168.1, 137.4, 137.0, 131.7, 126.2, 123.3, 121.6, 118.5, 113.5, 112.8, 109.8, 60.1, 44.6, 25.6, 14.5, 14.4; HRMS (ESI) (M+H) 274.1446; Calculated for $C_{16}H_{20}NO_3$ 274.1443.

4.4. General procedure for Henry reaction. In a round bottom flask fitted with a reflux condenser were taken suitable aldehyde **3** or **12** (1 eq.), ammonium acetate (2 eq.), nitromethane (excess) and acetic acid (4-5 drops). The contents were refluxed together for 3 h. After 3 h, excess nitromethane was removed under reduced pressure and the residue was extracted with ethyl acetate. Evaporation of the solvent under reduced pressure followed by column chromatography gave the corresponding nitro-olefins **4** and **13**.

4.4.1. (E)-4-(3-(2-Nitrovinyl)-1H-indol-1-yl)butan-1-ol (**4**)**.** Viscous oil (87%); FTIR (KBr cm^{-1}) 3400, 1616, 1309, 1165; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.24 (1H, d, *J*=13.2 Hz, ArC*H*=CH-), 7.72-7.77 (2H, m, Ar*H*, -C*H*NO$_2$), 7.57 (1H, s, Ar*H*), 7.42 (1H, d, *J*=7.4 Hz, Ar*H*), 7.27-7.37 (2H, m, Ar*H*), 4.21 (2H, t, *J*=7Hz, -NC*H*$_2$), 3.65 (2H, t, *J*=6.2 Hz, -OC*H*$_2$), 2.23 (1H, brs, exch., -O*H*), 1.99 (2H, quint, *J*=7.3 Hz, -C*H*$_2$-), 1.58 (2H, quint, *J*=6.1 Hz, -C*H*$_2$-); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 137.5, 136, 133.5, 131.4, 125.4, 123.6, 122.2, 120.5, 110.6, 107.9, 61.8, 46.8, 29.5, 26.3; Found: C 64.21, H 6.73, N 9.51, $C_{14}H_{16}N_2O_3$ requires: C 64.61, H 6.15, N 10.76 %.

4.4.2. (E)-3-(3-(2-Nitrovinyl)-1H-indol-1-yl)propan-1-ol (**13**). Yellow solid (88%); mp 72-75 °C; FTIR (KBr cm^{-1}) 3592, 3389, 1611, 1553, 1369, 1044; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.25 (1H, d, *J*=13.4 Hz, ArC*H*=CH-), 7.73-7.77 (2H, m, Ar*H*, -C*H*NO$_2$), 7.63 (1H, s, Ar*H*), 7.47 (1H, d, *J*=7.6 Hz, Ar*H*), 7.29-7.38 (2H, m, Ar*H*), 4.35 (2H, t, *J*=6.7 Hz, -NC*H*$_2$), 3.63 (2H, t, *J*=5.7 Hz, -OC*H*$_2$), 2.09 (2H, quint, *J*=6.7, 5.7Hz, -C*H*$_2$-), 1.88 (1H, brs, exch, -O*H*); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 137.8, 136.6, 133.7, 131.8, 125.6, 123.9, 122.5, 120.7, 110.8, 108.2, 58.7, 43.3, 31.9; HRMS (ESI) (M+H) 247.1082; Calculated for $C_{13}H_{15}N_2O_3$ 247.1082.

4.5. General procedure for oxidation using IBX. To a solution of suitable hydroxyl compound **4**/**8**/**13**/**16**/**19**/**22** (1 eq.) in dry ethyl acetate (20 ml) was added *o*-iodoxybenzoic acid (1.5 eq.) under inert atmosphere. The contents were refluxed for 8 h and the reaction was followed by TLC. After completion of reaction, the reaction mixture was filtered through celite bed on a sintered funnel and washed with ethyl acetate. Evaporation of the solvent under reduced pressure followed by column chromatography gave corresponding aldehydes **5**/**9**/**14**/**17**/**20**/**23**.

4.5.1. (E)-4-(3-(2-Nitrovinyl)-1H-indol-1-yl)butanal (**5**)**.** Yellow solid (95%); mp 81 °C; FTIR (KBr cm^{-1}) 2733, 1718, 1554, 1379, 746; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.74 (1H, s, -C*H*O), 8.21 (1H, d, *J*=13.2 Hz, ArC*H*=CH-), 7.70-7.74 (2H, m, Ar*H*, -C*H*NO_2), 7.52 (1H, s, Ar*H*), 7.43 (1H, d, *J*=8.2 Hz, Ar*H*), 7.27-7.37 (2H, m, Ar*H*), 4.22 (2H, t, *J*=7.1Hz, -NCH_2), 2.51 (2H, t, *J*=6.8 Hz, -CH_2CHO), 2.21 (2H, quint, *J*=7.1 Hz, -CH_2-); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 200.2, 137.4, 135.4, 133.1, 132.1, 125.4, 123.9, 122.4, 120.7, 110.6, 108.3, 45.8, 40.2, 22.3; Found: C 65.19, H 5.87, N 10.94, $C_{14}H_{14}N_2O_3$ requires: C 65.11, H 5.42, N 10.85%.

4.5.2. (E)-Ethyl 3-(1-(4-oxobutyl)-1H-indol-3-yl)acrylate (**9**). White solid (96%); mp 61 °C; FTIR (KBr cm^{-1}) 2756, 1731, 1667, 1651, 1538, 1186; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.73 (1H, s, -C*H*O), 7.83-7.91 (2H, m, Ar*H*, ArC*H*=CH-), 7.21-7.37 (4H, m, Ar*H*), 6.40 (1H, d, *J*=15.6 Hz, -C*H*COOEt), 4.16-4.29 (4H, m, -NCH_2, -C$H_2$$CH_3$), 2.46 (2H, t, *J*=6.8 Hz, -CH_2CHO), 2.18 (2H, quint, *J*=6.8 Hz, -CH_2-), 1.35 (3H, t, *J*=7.1 Hz, -CH_2CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 200.5, 168.1, 137.6, 137.1, 131.8, 125.9, 122.9, 121.2, 120.6, 112.7, 112.2, 109.9, 60.0, 45.3, 40.3, 22.3, 14.4; HRMS (ESI) (M+H) 286.1444; Calculated for $C_{17}H_{20}NO_3$ 286.1443.

4.5.3. (E)-3-(3-(2-Nitrovinyl)-1H-indol-1-yl)propanal (**14**). Yellow solid (94%); mp 112-115 °C; FTIR (KBr cm^{-1}) 2727, 1714, 1612, 1526, 1389; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.79 (1H, s, -C*H*O), 8.19 (1H, d, *J*=13.3 Hz, ArC*H*=CH-), 7.65-7.75 (3H, m, Ar*H*, -C*H*NO_2), 7.29-7.42 (3H, m, Ar*H*), 4.51 (2H, t, *J*=6.2 Hz, -NCH_2), 3.09 (2H, t, *J*=6.2 Hz, -CH_2CHO); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 198.6, 137.2, 136.5, 133.2, 132.1, 125.5, 123.9, 122.5, 120.8, 110.2, 108.3, 43.07, 39.5; HRMS (ESI) (M+H) 245.0930; Calculated for $C_{13}H_{13}N_2O_3$ 245.0926.

4.5.4. (E)-Ethyl 3-(1-(3-oxopropyl)-1H-indol-3-yl)acrylate (**17**). Yellow oil (95%); FTIR (KBr cm^{-1}) 2726, 1693, 1616, 1381, 1157; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.80 (1H, s, -C*H*O), 7.81-7.92 (2H, m, Ar*H*, ArC*H*=CH-), 7.40 (1H, s, Ar*H*), 7.23-7.34 (3H, m, Ar*H*), 6.41 (1H, d, *J*=15.9 Hz, -C*H*COOEt), 4.40 (2H, t, *J*=6.8 Hz, -NCH_2), 4.24 (2H, q, *J*=7.1 Hz, -C$H_2$$CH_3$), 2.81 (2H, t, *J*=6.8 Hz, -CH_2CHO), 1.33 (3H, t, *J*=7.1 Hz, -CH_2CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 184.4, 172.3, 138.1, 137.1, 125.3, 124.0, 122.9, 122.1, 118.2, 109.9, 60.7, 46.1, 30.7, 24.9; HRMS (ESI) (M+Na): 294.1104 Calculated for $C_{16}H_{17}NO_3Na$ 294.1106.

4.5.5. (E)-4-(3-Styryl-1H-indol-1-yl)butanal (**20**). Yellow oil (92%); FTIR (ATR cm^{-1}) 2932, 2724, 1714, 1627, 1456, 1360, 1165, 953, 737; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.74 (1H, s, -C*H*O), 8.01 (1H, d, *J*=7.6 Hz, Ar*H*), 7.54 (2H, d, *J*=7.7 Hz, Ar*H*), 7.10-7.46 (9H, m, Ar*H*), 4.17 (2H, t, *J*=6.7 Hz, -NCH_2), 2.43 (2H, t, *J*=6.7 Hz, -CH_2CHO), 2.16 (2H, quint., *J*=6.7 Hz, -CH_2-); (signals of *Z* isomer were seen in very small proportion); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 200.7, 138.5, 136.9, 128.5, 127.1, 126.5, 126.2, 125.7, 125.6, 125.1, 122.3, 121.3, 120.3, 120.1, 114.4, 109.6, 45.1, 40.5, 22.4; HRMS (ESI) (M+H): 290.1549; Calculated for $C_{20}H_{20}NO$ 290.1545.

4.5.6. (Z)-4-(3-(Prop-1-en-1-yl)-1H-indol-1-yl)butanal (**23**). Viscous oil (95%); FTIR (ATR cm^{-1}) 2928, 2873, 1716, 1611, 1466, 1366, 1169, 1075, 742; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.82 (1H, s, -C*H*O), 7.69 (1H, d, *J*=7.7 Hz, Ar*H*), 7.07-7.33 (4H, m, Ar*H*), 6.67 (1H, d, *J*=11.5 Hz,

ArC*H*=CH-), 5.69-5.80 (1H, m, -CH=C*H*Me), 4.13 (2H, t, *J*=7.2 Hz, -NCH_2), 2.43 (2H, t, *J*=7.1 Hz, -CH_2CHO), 1.96-2.14 (5H, m, -CH_2-, -CH_3); (signals of *E* isomer were seen in very small proportion); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 200.3, 137.5, 135.5, 133.2, 132.1, 125.5, 123.9, 122.5, 120.7, 110.6, 108.3, 45.9, 40.3, 22.3, 14.5; HRMS (ESI) (M+H): 228.1384; Calculated for $C_{15}H_{18}NO$ 228.1388.

*4.6. General procedure for formation of oximes **5a** and **9a**.* In a round bottom flask, hydroxyl amine hydrochloride (1.5 eq.) and sodium acetate (1.5 eq.) were added to a solution of suitable aldehyde **5** or **9** (1 eq.) in dry toluene under inert atmosphere. The reaction mixture was refluxed at 110 °C. After the completion of reaction, toluene was evaporated under reduced pressure and the residue was extracted with ethyl acetate. Evaporation of the solvent under reduced pressure gave the corresponding oxime **5a** or **9a**.

4.6.1. (E)-4-(3-((E)-2-Nitrovinyl)-1H-indol-1-yl)butanal oxime (**5a**). Sticky solid (94%); FTIR (KBr cm^{-1}) 3446, 1624, 1529, 1373, 1165; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.25 (1H, d, *J*=15.4 Hz ArC*H*=CH-), 7.77 (2H, m, Ar*H*), 7.62 (1H, d, *J*=15.8 Hz, -C*H*NO_2), 7.30-7.46 (4H, m, Ar*H*), 4.39 (2H, m, -NCH_2), 2.22-2.35 (4H, m, -CH_2-, -CH_2N-), 1.61 (1H, brs, exch, -O*H*); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 135.1, 134.3, 132.8, 124.3, 124.3, 122.8, 122.5, 120.9, 120.2, 110.3, 110.1, 45.1, 25.6, 14.6; HRMS (ESI) (M+H) 274.1196; Calculated for $C_{14}H_{16}N_3O_3$ 274.1192.

4.6.2. (E)-Ethyl 3-(1-((Z)-4-(hydroxyimino)butyl)-1H-indol-3-yl)acrylate (**9a**). Viscous oil (96%); FTIR (KBr cm^{-1}) 3466, 1703, 1622, 1467, 1278, 1168; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.76 (1H, brs, exch, -O*H*), 7.86 (2H, m, ArC*H*=CH-, Ar*H*), 7.17-7.40 (5H, m, Ar*H*), 6.38 (1H, d, *J*=15.6 Hz, -C*H*COOEt), 4.24 (2H, q, *J*=6.4 Hz, -C$H_2$$CH_3$) 4.10 (2H, t, *J*=6.6 Hz, -NCH_2), 2.38 (1H, q, *J*=6.9 Hz, -CH*H*=N-), 2.17 (1H, q, *J*=6.3 Hz, -CH*H*=N-), 2.01 (2H, quint, *J*=6.9 Hz, -CH_2-), 1.33 (3H, t, *J*=6.3 Hz, -CH_2CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 168.4, 138.1, 137.3, 132.5, 126.1, 122.9, 121.2, 120.6, 119.3, 112.5, 112.1, 110.1, 60.1, 58.9, 42.9, 32.2, 14.4; HRMS (ESI) (M+H) 301.1556; Calculated for $C_{17}H_{21}N_2O_3$ 301.1552.

4.7. 2-Nitro-5,6-dihydro-4H-indolo[3,2,1-de][1,5]naphthyridine (**6**). In a round bottom flask equipped with a reflux condenser, hydroxyl amine hydrochloride (0.2 g, 2.9 mmol) and sodium acetate (0.23 g, 2.9 mmol) were added to a solution of (*E*)-4-(3-(2-nitrovinyl)-1*H*-indol-1-yl)butanal (**5**) (0.5 g, 1.9 mmol) in dry toluene under inert atmosphere. The reaction mixture was refluxed and the reaction was monitored to completion by TLC. After 72 h, toluene was evaporated under reduced pressure and the residue was extracted with ethyl acetate. Evaporation of the solvent under reduced pressure followed by column chromatography (1:9, EA: Hexane) gave compound **6** as a dark yellow viscous oil (0.32 g, 67%); FTIR (KBr cm^{-1}) 1598, 1345, 1170; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.30 (1H, s, Ar*H*), 7.88 (1H, d, *J*=5.5Hz, Ar*H*), 7.25-7.41 (3H, m, Ar*H*), 4.22 (2H, t, *J*=6.7Hz, -NCH_2), 3.61 (2H, t, *J*=5.8Hz, ArCH_2), 2.18 (2H, quint, *J*=7.1, -CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 134.3, 127.6, 124.0, 122.2, 119.8, 118.1, 115.4, 110.0, 45.1, 25.5, 14.5; HRMS (ESI) (M+Na): 276.0748; Calculated for $C_{14}H_{11}N_3O_2Na$ 276.0749.

4.8. (E)-Ethyl 3-(1H-indol-3-yl)acrylate (**7**). Prepared according to literature procedure[10] (Mp 117-119 °C).

4.9. (E)-Ethyl 3-(1-(3-cyanopropyl)-1H-indol-3-yl)acrylate (**11**). To a solution of DMSO (10 ml) taken in a round bottom flask was added KOH (1.04 g, 18.6 mmol) and the solution was stirred at room temperature for 15 min. (*E*)-Ethyl 3-(1*H*-indol-3-yl)acrylate **(7)** (2 g, 9.3 mmol) was then added and the mixture was further stirred for 1 h, after which 4-bromobutanenitrile (1.43 ml, 13.9 mmol) was added dropwise to the reaction mixture. The reaction was monitored by TLC to completion within 5 h. The reaction mixture was then quenched by adding water and was extracted with ethyl acetate. Evaporation of the solvent under reduced pressure followed by column chromatography (1.5:8.5, EA: Hexane) gave compound **11** as a white solid (2.30 g, 88%); mp 77 °C; FTIR (KBr cm^{-1}) 2300, 1693, 1625, 1176, 1154; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.84-7.92 (2H, m, Ar*H*, ArC*H*=CH-), 7.22-7.36 (4H, m, Ar*H*), 6.43 (1H, d, *J*=15.9 Hz, -C*H*COOEt), 4.22-4.29 (4H, m, -NCH_2, -CH_2CH$_3$), 2.14-2.27 (4H, m, -CH_2-, -CH_2CN), 1.34 (3H, t, *J*=7.1 Hz, -CH$_2$CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 168.8, 137.4, 137.1, 131.7, 126.2, 123.3, 121.6, 120.8, 118.5, 113.5, 112.8, 109.8, 60.1, 44.6, 25.6, 14.5, 14.4; HRMS (ESI) (M+Na) 305.1267; Calculated for $C_{17}H_{18}N_2O_2Na$ 305.1266.

4.10. General procedure for Wittig reaction. To a solution of suitable Wittig salt benzylbromotriphenylphosphorane or ethyliodotriphenylphosphorane (2 eq.) in dry THF (20 ml) was added 1.6 M *n*-butyl lithium (3 eq.) at 0 °C under nitrogen atmosphere. A yellow colored anion was formed after which a solution of 1-(4-hydroxybutyl)-1*H*-indole-3-carbaldehyde **(3**) (0.5 g, 2.3 mmol) in dry THF (5 ml) was added dropwise to the above mixture. After the completion of reaction, the mixture was quenched by addition of saturated ammonium chloride solution and then extracted with ethyl acetate. Evaporation of the solvent under reduced pressure followed by column chromatography gave the corresponding olefin **19** or **22**.

4.10.1. (E)-4-(3-Styryl-1H-indol-1-yl)butan-1-ol (**19**). Pale yellow viscous oil (0.57 g, 85%); FTIR (ATR cm^{-1}) 3363, 2934, 1633, 1494, 1334, 1164, 740; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.01 (1H, d, *J*=7.1 Hz, Ar*H*), 7.53 (2H, d, *J*=7.7 Hz, Ar*H*), 7.21-7.45 (8H, m, Ar*H*), 7.12 (1H, d, *J*=16.7 Hz, =C*H*Ph), 4.13 (2H, t, *J*=7.2 Hz, -NCH_2), 3.61 (2H, t, *J*=6.7 Hz, -CH_2OH), 1.92 (2H, quint. *J*=7.2 Hz, -CH_2-), 1.51-1.62 (3H, m, -CH_2-, -O*H*); (signals of *Z* isomer were seen in very small proportion); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 138.7, 137.0, 128.6, 127.4, 126.4, 126.3, 125.7, 124.8, 122.2, 121.6, 120.3, 120.1, 114.1, 109.7, 62.2, 46.1, 29.9, 26.6; HRMS (ESI) (M+H): 292.1708; Calculated for $C_{20}H_{22}NO$ 292.1701.

4.10.2. (Z)-4-(3-(Prop-1-en-1-yl)-1H-indol-1-yl)butan-1-ol (**22**). Viscous oil (0.47 g, 89%); FTIR (ATR cm^{-1}) 3364, 2927, 1534, 1469, 1281, 1072, 960, 740; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.68 (1H, d, *J*=7.7 Hz, Ar*H*), 7.12-7.35 (4H, m, Ar*H*), 6.66 (1H, d, *J*=12.4 Hz, ArC*H*=CH-), 5.74 (1H, m, -CH=C*H*Me), 4.16 (2H, t, *J*=6.6 Hz, -NCH_2), 3.60 (2H, t, *J*=6.6 Hz, -CH_2OH), 1.83-1.97 (5H, m, -CH_2-, -CH_3), 1.55 (3H, m, -CH_2-, -O*H*); (signals of *E* isomer were seen in very small proportion); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 135.4, 127.9, 126.2, 122.6, 121.7, 120.4, 119.2,

119.1, 112.4, 109.1, 62.2, 46.1, 29.8, 26.6, 15.6; HRMS (ESI) (M+H): 230.1554; Calculated for $C_{15}H_{20}NO$ 230.1545.

*4.11. General procedure for canthine derivatives **6**, **10**, **15**, **18**, **21** and **24***. To a solution of suitable aldehyde **5**/**9**/**14**/**17**/**20**/**23** (1 eq.) in toluene was added hydrazine hydrate in excess (1 ml). The reaction mixture was then refluxed for 60 h. Toluene was then removed under reduced pressure and the residue was extracted with ethyl acetate. Evaporation of the solvent under reduced pressure followed by column chromatography gave the corresponding canthine derivative.

4.11.1. Ethyl 5,6-dihydro-4H-indolo[3,2,1-de][1,5]naphthyridine-2-carboxylate (**10**). Viscous oil (65%); FTIR (KBr cm^{-1}) 1693, 1614, 1525, 1150, 1094; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.32 (1H, s, Ar*H*), 7.75-7.78 (1H, m, Ar*H*), 7.25-7.42 (3H, m, Ar*H*), 4.25 (2H, t, *J*=7.2 Hz, -NCH_2), 4.12 (2H, q, *J*=7.1 Hz, -CH_2CH$_3$), 2.32 (2H, t, *J*=6.6 Hz, ArCH_2), 2.19 (2H, quint, *J*=6.6 Hz, -CH_2-), 1.23 (3H, t, *J*=7.1 Hz, -CH$_2$CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 167.9, 137.1, 136.5, 131.1, 126.3, 123.5, 121.8, 121.0, 116.7, 114.1, 113.4, 109.2, 60.1, 42.1, 24.9, 18.9, 14.3; HRMS (ESI) (M+H) 281.1290; Calculated for $C_{17}H_{17}N_2O_2$ 281.1290.

4.11.2. 2-Nitro-4,5-dihydrobenzo[b]pyrido[2,3,4-gh]pyrrolizine (**15**). Yellow solid (62%); mp 120-122 °C; FTIR (KBr cm^{-1}) 1612, 1529, 1389; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.57 (1H, s, Ar*H*), 8.12 (1H, d, *J*=7.7 Hz, Ar*H*), 7.42 (1H, t, *J*=7.2 Hz, Ar*H*), 7.25-7.32 (1H, m, Ar*H*), 7.17-7.21 (1H, m, Ar*H*), 4.20 (2H, t, *J*=5.8 Hz, -NCH_2), 3.06 (2H, t, *J*=6.2 Hz, ArCH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 137.3, 132.9, 132.5, 125.6, 124.2, 122.7, 120.8, 110.3, 108.7, 45.0, 25.4; HRMS (ESI) (M+Na) 262.0594; Calculated for $C_{13}H_9N_3O_2Na$ 262.0592.

4.11.3. Ethyl 4,5-dihydrobenzo[b]pyrido[2,3,4-gh]pyrrolizine-2-carboxylate (**18**). Pale yellow oil (56%); FTIR (KBr cm^{-1}) 1692, 1615, 1158; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.61 (1H, s, Ar*H*), 8.11 (1H, d, *J*=7.7 Hz, Ar*H*), 7.24-7.26 (1H, m, Ar*H*), 7.08-7.19 (2H, m, Ar*H*), 4.21-4.28 (4H, m, -NCH_2, -CH_2CH$_3$), 3.65 (2H, t, *J*=7.2 Hz, ArCH_2), 1.22 (3H, t, *J*=7.2 Hz, -CH$_2$CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 167.9, 137.1, 136.5, 131.1, 126.3, 123.5, 121.8, 121.0, 116.7, 114.1, 113.4, 109.2, 60.1, 42.1, 18.9, 14.3; HRMS (ESI) (M+H): 267.1132; Calculated for $C_{16}H_{15}N_2O_2$ 267.1133.

4.11.4. 2-Phenyl-5,6-dihydro-4H-indolo[3,2,1-de][1,5]naphthyridine (**21**). Thick oil (65%); FTIR (ATR cm^{-1}) 2933, 2838, 1697, 1597, 1244, 1026, 827; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.12 (1H, d, *J*=7.7 Hz, Ar*H*), 7.86 (1H, s, Ar*H*), 7.49-7.54 (1H, m, Ar*H*), 7.39-7.45 (3H, m, Ar*H*), 7.25-7.32 (2H, m, Ar*H*), 7.17-7.21 (2H, m, Ar*H*), 4.20 (2H, t, *J*=5.8 Hz, -NCH_2), 3.06 (2H, t, *J*=6.2 Hz, ArCH_2), 2.30 (2H, quint, *J*=5.7 Hz, -CH_2-); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 155.4, 140.8, 139.3, 132.4, 130.4, 129.9, 127.6, 126.4, 122.5, 121.4, 121.3, 120.3, 119.9, 119.0, 118.6, 108.8, 40.7, 22.6, 21.8; HRMS (ESI) (M+H): 285.1400; Calculated for $C_{20}H_{17}N_2$ 285.1392.

4.11.5. 2-Methyl-5,6-dihydro-4H-indolo[3,2,1-de][1,5]naphthyridine (**24**). Thick oil (68%); FTIR (ATR cm^{-1}) 2968, 2865, 1690, 1439, 1175, 773; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.27 (1H,

d, *J*=7.1 Hz, Ar*H*), 7.96 (1H, s, Ar*H*), 7.29-7.45 (3H, m, Ar*H*), 4.53 (2H, t, *J*=6.6 Hz, -NCH_2), 2.97 (2H, t, *J*=6.6 Hz, ArCH_2), 2.52 (3H, s, -CH_3), 2.18 (2H, quint, *J*=6.7 Hz, -CH_2-); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 154.4, 148.5, 137.9, 135.4, 130.1, 127.4, 120.8, 119.3, 117.9, 109.2, 104.9, 46.9, 32.1, 26.6, 23.2; HRMS (ESI) (M+H): 223.1239; Calculated for $C_{!5}H_{15}N_2$ 223.1235.

4.12. Cytotoxicity test. The cytotoxicity of the compounds was measured at 24 and 48 h intervals in triplicate using a standard MTT assay against human cervical cell line (HeLa). The average cytotoxicity data and the associated standard deviation (SD) obtained were then used to calculate cell viability.[11]

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