Synthesis and in Vitro Cytotoxic Activity of Some Novel β-Carbolines bearing pyrrol-2-one moiety

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ABSTRACT

β-carboline are reported to have anticancer activities on cultured cancer cell lines.To further enhance the cytotoxic potency, a series of novel 5-substituted β-carboline derivatives have been synthesized from 5-Amino-4-(1H-indol-3-yl)-1,3-dihydro-pyrrol-2-one via Bischler-Napieralski reaction . The newly synthesized compounds were characterized by spectral studies. All compounds were evaluated for in vitro cytotoxic activity against three human cancer cell lines(HeLa,HepG2 and A431)using MTT assay.

Keywords: Synthesis, β-carbolines, Bischler-Napieralski reaction, cytotoxic activity, MTT assay.

INTRODUCTION

β-carbolines display a broad spectrum of pharmacological properties, including anxiolytic, hypnotic, sedative, anticonvulsant, antiviral, antiparasitic, anticancer, and antimicrobial activities.[1-7] Among the activities presented, the anticancer activity has received special attention, and several studies on structure-activity relationship of β-carbolines have focused their anticancer activities.[8-16]

In view of biological significance of β-carbolines, it was planned to synthesize some new derivatives containing pyrrol-2-one moiety to get the more potent compounds and evaluate their potential *in vitro* cytotoxic activity against three human cancer cell lines including HeLa(Cervical cancer),HepG2(Liver cancer) and A431(Skin cancer).In addition, the structure-activity relationship studies of substitution preferences for enhanced cytotoxic activity have been elucidated.

Many papers have reported the synthesis and biological studies of β-carboline derivatives.[17-23] Generally there are two ways to synthesize β-carbolines.One is through the Bischler-Napieralski reaction[24] and the other through Pictet-Spengler reaction[25]. We selected the Bischler-Napieralski cyclisation in our study.

EXPERIMENTAL SECTION

General

All chemical reagents and solvents used in this study were purchased from Sigma-Aldrich Co.Melting points were determined using a digital melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Ultrashield 500 instrument using $CDCl_3$ as solvent (unless otherwise stated) and tetramethylsilane as internal standard.The purity of the compounds was checked by thin layer chromatography (TLC) on silica gel plates.

Chemistry

4-amino-5-(1H-indol-3-yl)-pyrrolidin-2-one was syntheiszed as per the literature procedure.[26]

5-amino-4-(1H-indol-3-yl)-1,3-dihydro-pyrrol-2-one was synthesized as per the literature procedure. 5-amino-4-(1H-indol-3-yl)-1,3-dihydro-pyrrol-2-one **(2)** was synthesized from

4-amino-5-(1H-indol-3-yl)-pyrrolidin-2-one **(1)** by oxidation using KMnO4 in DMF.

Synthesis of 5-amino-4-(1H-indol-3-yl)-1,3-dihydro-pyrrol-2-one (2)

To the solution of 4-amino-5-(1H-indol-3-yl)-pyrrolidin-2-one (5.58 mmol) in DMF (10 ml), potassium permanganate(2.53 mmol) was added at $0^{o}C$ and then left stirring overnight.The mixture was poured in to water to collect the top deposit. Recrystallization was done by using methanol.

Yield:76%,Brown solid, m.p 232 - 235^{o}C

^{1}HNMR Spectra ($CDCl_3$): δ (ppm)10.06 (–NH),8.02(-CONH), 2.04($-NH_2$),7.08- 8.92 (H, indole ring),2.91 ($-CH_2$). ^{13}CNMR Spectra ($CDCl_3$): δ (ppm) 167.11(-CONH),101.2-135.33 (C, indole ring),40.12 ($-CH_2$), 91.05(C,pyrrole ring), 141.14($C-NH_2$, pyrrole ring).

General Procedure for the synthesis of amides of 5-Amino-4-(1H-indol-3-yl)-1,3-dihydro-pyrrol-2-one 3(a-f)

To the solution of **(2)** (1.26 mmol) in CH_3CN (20 ml), various acid chlorides were added (1.37 mmol) and the resultant mixture was refluxed with triethyl amine (2 ml) at $90^{o}C$ for 4 Hrs in a 250 mL single-necked, round-

bottomed flask. Evaporation to dryness under reduced pressure yielded crude solid residues. Pure compounds were obtained as brown solids by column chromatography using MeOH-$CHCl_3$ as eluent.

N-[3-(1H-Indol-3-yl)-5-oxo-4,5-dihydro-1H-pyrrol-2-yl]-acetamide (3a)

Yield:71%,Brown solid, m.p 295 - 298^oC

^{1}HNMR Spectra ($CDCl_3$): δ (ppm)10.12 (–NH,indole ring),8.06(-CONH), 8.04(-CONH,pyrrole ring),7.21- 8.74 (H,indole ring),3.12(-CH_2), 1.93(-CH_3) ^{13}CNMR Spectra ($CDCl_3$): δ (ppm) 167.14(-CONH),167.27(-CONH,pyrrole ring),101.2-136.23 (C,indole ring), 94.13(C,pyrrole ring), 127.24(C-NH_2, pyrrole ring),39.37(-CH_2), 19.05(-CH_3).

N-[3-(1H-Indol-3-yl)-5-oxo-4,5-dihydro-1H-pyrrol-2-yl]-benzamide (3b)

Yield:76%, Brown solid, m.p 251 - 254^oC

^{1}HNMR Spectra ($CDCl_3$): δ (ppm)10.07 (–NH,indole ring),8.06(-CONH), 8.03(-CONH,pyrrole ring),7.13- 9.15 (H,indole ring), 7.37-8.11(H,phenyl ring),2.96 (-CH_2).^{13}CNMR Spectra ($CDCl_3$): δ (ppm) 167.34(-CONH,pyrrole ring),164.82(-CONH),102.31-137.03(C,indole ring), 125.05-133.34(C,phenyl ring),94.17(C,pyrrole ring), 126.84(C-NH_2,pyrrole ring),40.04(-CH_2).

4-Hydroxy-N-[3-(1H-indol-3-yl)-5-oxo-4,5-dihydro-1H-pyrrol-2-yl]-benzamide (3c)

Yield:69%, Brown solid, m.p 255- 258^oC

^{1}HNMR Spectra ($CDCl_3$): δ (ppm)10.11 (–NH, indole ring),8.02(-CONH), 8.01(-CONH,pyrrole ring),7.08- 9.22 (H,indole ring),6.79-7.91(H,phenyl ring),2.81 (-CH_2),5.02 (-OH) ^{13}CNMR Spectra ($CDCl_3$): δ (ppm) 167.14(-CONH,pyrrole ring),163.87(-CONH),101.55-136.11 (C, indole ring),115.05-160.34(C,phenyl ring),93.17(C,pyrrole ring), 126.74(C-NH_2 of pyrrole ring),40.63 (-CH_2).

N-[3-(1H-Indol-3-yl)-5-oxo-4,5-dihydro-1H-pyrrol-2-yl]-4-methyl-benzamide (3d)

Yield:72%, Brown solid, m.p 286- 289^oC

^{1}HNMR Spectra ($CDCl_3$): δ (ppm)10.08 (–NH, indole ring),8.03(-CONH), 8.04(-CONH,pyrrole ring),7.13- 9.41 (H, indole ring),7.19-7.91(H,phenyl ring),2.75 (-CH_2),2.31 (-CH_3) ^{13}CNMR Spectra ($CDCl_3$): δ (ppm) 167.03(-CONH, pyrrole ring),164.44(-CONH),102.23-135.41 (C, indole ring), 125.05-140.34(C,phenyl ring), 93.57(C,pyrrole ring), 126.62(C-NH_2 of pyrrole ring),40.11 (-CH_2), 20.82 (-CH_3).

N-[3-(1H-Indol-3-yl)-5-oxo-4,5-dihydro-1H-pyrrol-2-yl]-4-methoxy-benzamide (3e)

Yield:75%, Brown solid, m.p 303- 306^oC

^{1}HNMR Spectra ($CDCl_3$): δ (ppm)10.02 (–NH, indole ring),8.02(-CONH), 8.03(-CONH, pyrrole ring),7.08-9.33 (H,indole ring),6.89-7.91(H,phenyl ring),2.78 (-CH_2),3.71(-OCH_3) ^{13}CNMR Spectra ($CDCl_3$): δ (ppm) 167.21(-CONH,pyrrole ring),164.29(-CONH),102.53-135.64 (C, indole ring), 125.05-165.34(C,phenyl ring), 93.45(C,pyrrole ring), 126.73(C-NH_2 of pyrrole ring),40.34 (-CH_2), 55.92 (-OCH_3).

N-[3-(1H-Indol-3-yl)-5-oxo-4,5-dihydro-1H-pyrrol-2-yl]-4-nitro-benzamide (3f)

Yield:73%, Brown solid m.p 294 - 297^oC

^{1}HNMR Spectra ($CDCl_3$): δ (ppm)10.08 (–NH, indole ring),8.02(-CONH), 8.04(-CONH, pyrrole ring),7.11-9.41 (H,indole ring), 8.19-8.41(H,phenyl ring),2.89 (-CH_2) ^{13}CNMR Spectra ($CDCl_3$): δ (ppm) 167.54(-CONH,pyrrole ring),164.11(-CONH),102.43-136.17 (C, indole ring), 123.05-152.34(C,phenyl ring), 40.53 (-CH_2).

***General Procedure for the synthesis of pyrrolo β-carbolines* 4(a-f)**

To a solution of **3(a-f)** (0.8 mmol) in acetonitrile (20 ml), $POCl_3$(3ml) was added carefully in a drop wise manner.The resultant mixture was heated to reflux for 3 Hrs. Acetonitrile and $POCl_3$ were removed under vacuum and the residues obtained were purified by chromatography using silica gel as the solid support and then eluted with MeOH-$CHCl_3$.

5-methyl-6H-pyrrolo[3,4-b]-β-carbolin-2-one (4a)

Yield:68%,Black solid m.p 311 - 314^oC

^{1}HNMR Spectra ($CDCl_3$): δ (ppm)10.08 (–NH),7.95(-CONH),7.24-7.63 (H, indole ring),3.45 (-CH_2), 2.52(-CH_3) ^{13}CNMR Spectra ($CDCl_3$): δ (ppm) 168.34(-CONH), 102.28-134.63 (C, indole ring), 121.32- 154.31 (C,pyridine ring),35.32 (-CH_2), 15.85(-CH_3).

5-phenyl-6H-pyrrolo[3,4-b]-β-carbolin-2-one (4b)

Yield:65%,Black solid, m.p 269 - 272^oC

^{1}HNMR Spectra ($CDCl_3$): δ (ppm)10.12 (–NH),8.21(-CONH),7.11-7.64 (H, indole ring),7.21-8.10(H, phenyl ring)3.37 (-CH_2) ^{13}CNMR Spectra ($CDCl_3$): δ (ppm) 167.42(-CONH), 101.83-136.21 (C, indole ring),127.18-140.16 (C, phenyl ring) 121.15- 154.08 (C,pyridine ring),34.8 (-CH_2).

5-(4-hydroxyphenyl)-6H-pyrrolo[3,4-b]-β-carbolin-2-one (4c)

Yield:68%,Black solid, m.p 277 - 280^{o}C

^{1}HNMR Spectra ($CDCl_3$): δ (ppm)10.14 (–NH),8.13(-CONH),7.03-7.53 (H, indole ring),6.92-7.87(H, phenyl ring)3.42 (-CH_2),5.14(-OH) ^{13}CNMR Spectra ($CDCl_3$): δ (ppm) 167.75 (-CONH), 102.79-135.14 (C, indole ring), 121.03- 154.12 (C,pyridine ring), 117.22- 156.12 (C,phenyl ring),35.32 (-CH_2).

5-(4-methylphenyl)-6H-pyrrolo[3,4-b]-β-carbolin-2-one (4d)

Yield:67%,Black solid, m.p 299 - 302^{o}C

^{1}HNMR Spectra ($CDCl_3$): δ (ppm)10.07 (–NH),8.04(-CONH),7.06-8.21 (H, indole ring), 7.02-7.76(H, phenyl ring),3.41 (-CH_2),2.37(-CH_3) ^{13}CNMR Spectra ($CDCl_3$): δ (ppm) 168.17 (-CONH), 102.34-135.16 (C, indole ring), 120.85- 154.31 (C,pyridine ring), 127.26- 137.45 (C,phenyl ring), 34.36 (-CH_2), 19.83(-CH_3)

5-(4-methoxyphenyl)-6H-pyrrolo[3,4-b]-β-carbolin-2-one (4e)

Yield:65%,Black solid, m.p 319 - 322^{o}C

^{1}HNMR Spectra ($CDCl_3$): δ (ppm)10.11 (–NH),8.03(-CONH),7.04-7.57 (H, indole ring),6.76-7.73(H,phenyl ring),3.43 (-CH_2),3.72(-OCH_3) ^{13}CNMR Spectra ($CDCl_3$): δ (ppm) 168.23(-CONH), 102.61-134.68 (C, indole ring),121.03-154.32(C, pyridine ring),114.37- 160.45(C, phenyl ring), 35.35 (-CH_2), 56.37(-OCH_3)

5-(4-nitrophenyl)-6H-pyrrolo[3,4-b]-β-carbolin-2-one (4f)

Yield:67%,Black solid, m.p 313 - 316^{o}C

^{1}HNMR Spectra ($CDCl_3$): δ (ppm)10.10 (–NH),8.02(-CONH),7.03-7.58 (H, indole ring),8.18-8.30(H, phenyl ring)3.36 (-CH_2),^{13}CNMR Spectra ($CDCl_3$): δ (ppm) 168.13(-CONH), 101.85-136.45 (C, indole ring),120.64-154.16(C,pyridine ring),124.21-147.10(C,phenyl ring),35.71 (-CH_2).

Biological activity

Cell lines and cell cultures

All the three human cancer cell lines(HeLa,HepG2 and A431) were obtained from National Centre for Cell Science (NCCS), Pune, and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). All cells were maintained at 37^{0}C, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

Cytotoxicity assay

In this study, the MTT assay was taken to assess the cytotoxicity of pyrrolo-β-carbolines on human cancer cell proliferation. MTT assay was first described by Mosmann (Mosmann, 1983). The method is based on the ability of a mitochondrial dehydrogenase from viable cells to cleave the tetrazolium rings of the pale yellow MTT, and form purple formazan crystals which are impermeable to cell membranes. The crystals can be solubilized by detergents. The number of living cells is directly proportional to the level of the formed formazan, which can be quantified photometrically.

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium with 5% FBS to give final density of 1x10^{5} cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^{0}C, 5% CO_2, 95% air and 100% relative humidity.

Pyrrolo-β-carbolines were dissolved dimethylsulfoxide (DMSO) and further diluted in serum free medium to produce five concentrations. One hundred microlitres per well of each concentration was added to plates to obtain final concentrations of 500, 250, 125 62.5 and 31.25 µg/ml. The final volume in each well was 200 µl and the plates were incubated at 37^{0}C, 5% CO_2, 95% air and 100% relative humidity for 48h. The medium containing without samples were served as control. Triplicate was maintained for all concentrations.

After 48h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^{0}C for 4 h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then absorbance was measured at 570 nm using micro plate reader. Experiments were repeated three times independently. The % cell inhibition was determined using the following formula.

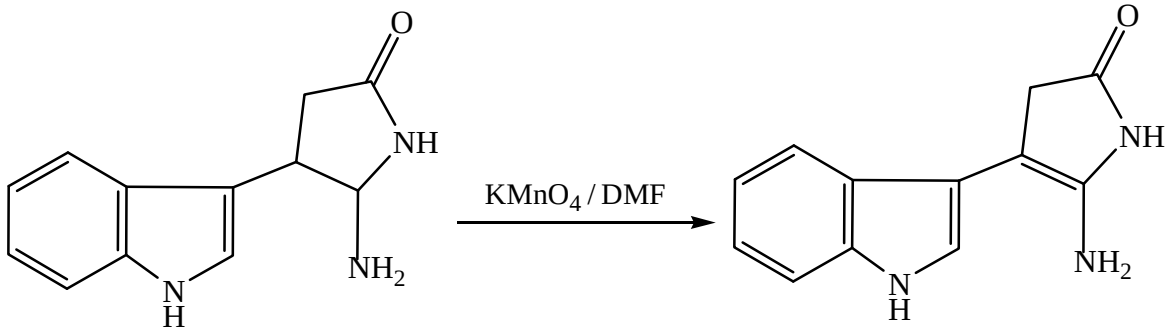
% cell Inhibition = 100- Abs (sample)/Abs (control) x100.

RESULTS AND DISCUSSION

Chemistry

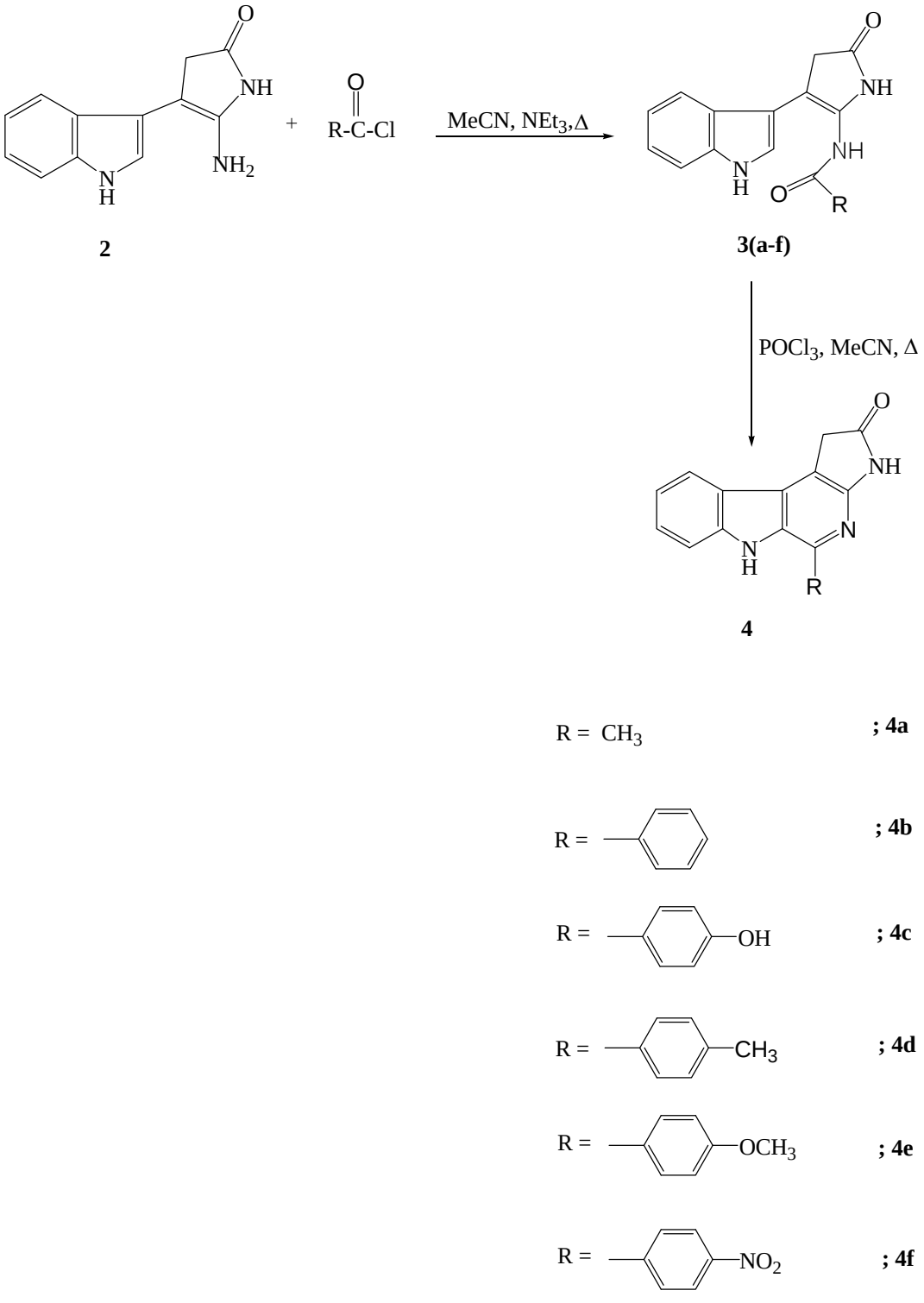
In this paper, we describe a two-step preparation of pyrrolo-β-carbolines **4(a-f)** using 5-amino-4-(1-*H*-indol-3-yl)-1,3-dihydro pyrrol-2-one **(2)** as the starting material. The later compound was prepared by the oxidation of 4-amino-5-(1H-indol-3-yl)-pyrrolidin-2-one **(1)** with $KMnO_4$ in the presence of DMF. (Scheme 1).

Starting from 5-amino-4-(1-*H*-indol-3-yl)-1,3-dihydro pyrrol-2-one **(2)** ,after reaction with various acid chlorides in the presence of acetonitrile and triethyl amine at refluxing,the corresponding amides obtained have been utilized as substrates in the cyclization step under Bischler-Napieralski reaction conditions.[27] (Scheme 2).



1 **2**

Scheme 1 : Synthesis of 5-amino-4-(1-*H*-indol-3-yl)-1,3-dihydro pyrrol-2-one



2 **3(a-f)** **4**

Scheme 2 : Synthetic route for pyrrolo-β-carbolines

3.3.2. In vitro cytotoxicity screening of pyrrolo-β-carbolines

The cytotoxic potential of all synthesized pyrrolo-β-carbolines was evaluated against a panel of three human cancer cell lines(HeLa,HepG2 and A431) using MTT(3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay to determine the drug concentration required to inhibit the growth of human cancer cells by 50% (IC_{50}).[28,29] The results of the IC_{50} data (μg/ml) for the anticancer activities are presented in **Table-1**.

In order to investigate the Structure activity relationship the intact β-carboline ring was reserved with an annulated pyrrolidin-2-one moiety, then different alkyl or aryl groups were added to position-5 of pyrrolo-β-carbolines.

The obtained results revealed that the most active compound was the β-carboline derivative **25** with a -CH_3 group at position-5 of β-carboline ring, which showed best inhibitory activity against all the three cancer cell lines HeLa(IC_{50} = 18 μg/ml),HepG2(IC_{50} = 24 μg/ml) and A431(IC_{50} = 41 μg/ml).

The compound **27**, having *p*-hydroxyphenyl substituent at position-5 of β-carboline ring caused inhibition on all three cancer cell lines HeLa(IC_{50} = 27 μg/ml),HepG2(IC_{50} = 33μg/ml) and A431(IC_{50} =77μg/ml).

Compounds **28** and **29 with** *p*-methylphenyl **and** *p*-methoxyphenyl substituents respectively are more potent than the compounds **26 and 30 with** phenyl **and** *p*-nitrophenyl substituents respectively at position-5 of β-carboline ring against HeLa and HepG2 cell lines. This showed that the introduction of electron-donating groups was effective to enhance the anticancer activity.

Table-1. In vitro cytotoxic activities of β-carboline derivatives (IC_{50}, μg/ml)[a,c]

Compound	HeLa[b]	HepG2[b]	A431[b]
25	18	24	41
26	61	54	157
27	27	33	73
28	34	40	86
29	39	45	92
30	52	68	165

[a] Cytotoxicity as IC_{50} for each cell line is the concentration of compound which reduced by 50% the optical density of treated cells with respect to untreated cells using the MTT assay.

[b] Cell lines include human cervical cancer cell lines (HeLa), human skin carcinoma cell lines (A431) and human liver cell carcinoma cell lines(HepG2)

[c] Data represent the mean values of three independent determinations.

CONCLUSION

A thorough literature studies have left no room for doubt that all the synthesized β-carbolines are new to the literature. Anticancer activity evaluation reveals that among the tested compounds, compound **25** showed best inhibitory activity against all the three cancer cell lines. Hence it can be suggested that compound **25** could be used as leads in the design and development of new anticancer drugs.

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