**Research Paper** www.ijcps.com

**International Journal of Chemical and Pharmaceutical Sciences**
2013, Sep., Vol. 4 (3)

ISSN: 0976-9390
IJCPS

# Synthesis and cytotoxic studies of a new series of pyridinoxymethylcoumarins

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**ABSTRACT**

4-Bromomethylcoumarins (1a-k) were reacted with 3-hydroxypyridines (2a-b) to yield pyridinoxymethylcoumarins (3a-r). The structure of all the synthesized compounds were confirmed by spectral studies and screened for their cytotoxic activities against Dalton's Ascitic Lymphoma (DAL) and Ehrlich Ascites Carcinoma (EAC) cell lines. Out of these, the compound (3o) was found to be the most cytotoxic against DAL cell line and the compound (3m) was found to be the most cytotoxic against EAC cell line.

**Keywords:** 4-Bromomethylcoumarins, Coumarins, Pyridine, Anticancer activity, Cytotoxicity

## 1. INTRODUCTION

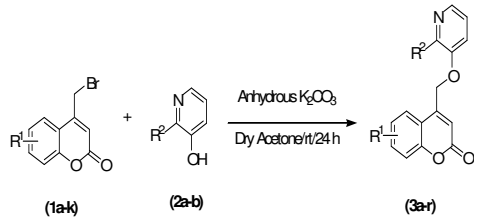
Pyridine derivatives are found to exhibit a wide spectrum of biological activities [1]. 2-Chloropyridine derivatives containing flavone moiety strongly inhibited telomerase with $IC_{50}$ value of 0.8±0.07µm by a modified TRAP assay [2]. 2-Amino-4-methyl-5-phenylsulfonylmethylpyridine exhibited potent fungicidal activity against *Alternaria tennis* [3]. Triazolyl pyridines were found to be potential focal adhesion kinase (FAK) inhibitor as antitumour agents against HCT116 cell lines with $IC_{50}$ values of 8.17µm [4]. 2,4,6-Triarylpyridines showed stronger topo II inhibitory activity than etoposide [5]. Benzimidazoyl and benzthiazolyl pyridines displayed the most pronounced activity against Melanoma cancer as MDA-MB-435 cell lines [6]. Pyridine derivatives inhibited kinase CDK5 with an inhibitory concentration of 160nM [7]. Molecular iodine catalysed, one pot synthesis of pyridine ring fused with imidazo ring has been reported [8].

Coumarins constitute an important class of benzopyrones exhibiting a broad range of biological activities [9]. One pot conversion of aminocoumarins to coumarinyl isothiocyanate with excellent yield has been described [10]. Synthesis of coumarins via Pechmann cyclisation using poly(4-vinylpyridine)-supported sulfuric acid as a reusable catalyst has been reported [11]. Quinoxalylcoumarins were synthesised from bromoacetyl coumarins under microwave irradiation [12]. Six new 4-hydroxycoumarin derivatives were synthesized and studied by computational methods-DFT (B3LYP) and force field methods (MM2 and OPLS), in order to optimize their geometry and calculate quantum-chemical properties and conformational analysis [13]. The molecular and crystal structure of 8-acetyl-7-[2-(1-morpholino)ethoxyl]-4-ethylchromen-2-one in solid state were analyzed by X-ray diffraction. The compound crystallized in the monoclinic space group $P2_1/c$ [14]. Phosphorohydrazine derivatives of coumarin demonstrated high *in vivo* antitumour activity against P388 leukemia [15]. Coumarin pyrazoline hybrids possessed the highest cytotoxicity against colorectal cell line HCT-116 with $IC_{50}$ value of 0.01µM [16]. Thiazolyl coumarin derivatives showed significant inhibition against Haemphilus influenzae with a MIC value of 15µM less than that of tetracycline [17]. Based on these reports, we considered the possibility of introducing pyridine moiety into the coumarin unit to design novel structures with enhanced cytotoxic activities.

### 1.1. Chemistry

4-Bromomethylcoumarins (1a-k) [18, 19] were synthesized by the Pechmann cyclisation of phenols with 4-bromoethylacetoacetate [20]. Various 4-bromomethylcoumarins (1a-k) reacted with 3-hydroxypyridines (2a-b) in the presence of

anhydrous $K_2CO_3$ to give 4-(pyridin-3-yloxymethyl)-*2H*-chromen-2-ones (3a-r) (Scheme 1). The numbering of the skeleton (3m) is shown in Figure 1.




$R^1$= 1a: 6-$CH_3$, 1b: 7-$CH_3$, 1c: 6-$OCH_3$, 1d: 6-Et, 1e: 6-i-Pr, 1f: 6-tert-Butyl, 1g: 6-Benzyl, 1h: 5,6-Benzo, 1i: 6-Cl, 1j: 6-Br, 1k: 6-F

$R^2$= 2a: $NH_2$, 2b: $CH_3$

**Scheme - 1: Synthesis of the pyridinoxymethylcoumarins**

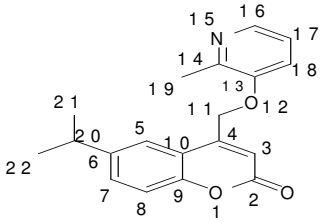



**Figure - 1: Numbering of the compound (3m)**

## 2. METHODS AND MATERIALS

### 2.1. MATERIALS

The melting points were determined by open capillary method using electric melting point apparatus and are uncorrected. The IR spectra (KBr disc) were recorded on a Shimadzu-8400S FT-IR Spectrophotometer. $^1$H NMR, $^{13}$C NMR and HSQC were recorded on Bruker 400MHz spectrometer by using $CDCl_3$ and DMSO-$d_6$ as a solvent and TMS as an internal standard. The chemical shifts are expressed in $\delta$ ppm. Elemental analyses (C, H, N) were conducted using the Elemental Analyser XBO. The mass spectra were recorded using Agilent-Single Quartz LC-MS. The purity of the compound was checked by TLC.

### 2.2. General procedure for synthesis of pyridinoxymethylcoumarins (3a-r)

A mixture of 3-hydroxypyridine (2a-b) (3.4 mmol) and anhydrous $K_2CO_3$ (1.38 g, 10 mmol) were stirred in 25mL of dry acetone for 30 min. 4-Bromomethylcoumarin (1a-k) (3.4 mmol) was added and stirring was continued for 24 h. The reaction mixture was concentrated to one fourth volume and poured on to crushed ice. The solid separated was filtered and washed with water. The crude product was dried and recrystalized from ethanol.

#### 2.2.1. 6-Methyl-4-((2-amino-pyridin-3-yloxy) methyl)-2*H*-chromen-2-one (3a)

Yield 95%; colorless solid; m.p.: 233-235 °C; IR (KBr): 1706 cm$^{-1}$ (lactone C = O), 3145 cm$^{-1}$, 3099 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ = 2.49 (s, 3H, $C_6$-$CH_3$), 5.38 (s, 2H, $OCH_2$), 5.95 (s, 2H, $NH_2$), 6.52 (t, 1H, $C_{17}$-H, $J_{1,2}$ = 5.2 Hz), 6.91 (s, 1H, $C_3$-H), 7.33-7.35 (m, 2H, $C_8$-H and $C_5$-H), 7.46 (d, 1H, $C_7$-H, $J_{1,2}$ = 8.8 Hz), 7.56 (d, 1H, $C_{18}$-H, $J_{1,2}$ = 4.8 Hz), 7.73 (s, 1H, $C_{16}$-H) ppm; Anal. Calcd for $C_{16}H_{14}N_2O_3$: C, 68.08: H, 5.0: N, 9.92. Found: C, 67.98: H, 4.97: N, 9.80.

#### 2.2.2. 7-Methyl-4-((2-amino-pyridin-3-yloxy) methyl)-2*H*-chromen-2-one (3b)

Yield 92%; colorless solid; m.p.: 221-223 °C; IR (KBr): 1726 cm$^{-1}$ (lactone C = O), 3139 cm$^{-1}$, 3074 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ = 2.48 (s, 3H, $C_7$-$CH_3$), 5.36 (s, 2H, $OCH_2$), 5.86 (s, 2H, $NH_2$), 6.50 (t, 1H, $C_{17}$-H, $J_{1,2}$ = 5.2 Hz), 6.80 (s, 1H, $C_3$-H), 7.20 (d, 1H, $C_5$-H, $J_{1,2}$ = 7.2 Hz), 7.26 (s, 1H, $C_8$-H), 7.29 (d, 1H, $C_6$-H, $J_{1,2}$ = 6.8 Hz), 7.55 (d, 1H, $C_{18}$-H, $J_{1,2}$ = 6 Hz), 7.76 (d, 1H, $C_{16}$-H, $J_{1,2}$ = 8 Hz) ppm; Anal. Calcd for $C_{16}H_{14}N_2O_3$: C, 68.08: H, 5.0: N, 9.92. Found: C, 67.99: H, 4.96: N, 9.81.

#### 2.2.3. 6-Methoxy-4-((2-amino-pyridin-3-yloxy) methyl)-2*H*-chromen-2-one (3c)

Yield 90%; colorless solid; m.p.: 195-197 °C; IR (KBr): 1722 cm$^{-1}$ (lactone C = O), 3149 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 3.84 (s, 3H, $OCH_3$), 4.72 (s, 2H, $NH_2$, $D_2O$ exchangeable), 5.21 (s, 2H, $OCH_2$), 6.59 (s, 1H, $C_3$-H), 6.62 (t, 1H, $C_{17}$-H, $J_{1,2}$ = 5.2 Hz), 6.97-7.00 (m, 2H, $C_5$-H & $C_{18}$-H), 7.14 (d, 1H, $C_7$-H, $J_{1,2}$ = 6.4 Hz), 7.32 (d, 1H, $C_8$-H, $J_{1,2}$ = 9.2 Hz), 7.74 (d, 1H, $C_{16}$-H, $J_{1,2}$ = 4.8 Hz) ppm; Anal. Calcd for $C_{16}H_{14}N_2O_4$: C, 64.42: H, 4.73: N, 9.39. Found: C, 64.03: H, 4.55: N, 9.23.

#### 2.2.4. 6-Ethyl-4-((2-amino-pyridin-3-yloxy) methyl)-2*H*-chromen-2-one (3d)

Yield 95%; colorless solid; m.p.: 194-196 °C; IR (KBr): 1706 cm$^{-1}$ (lactone C = O), 3161 cm$^{-1}$, 3066 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 1.27 (t, 3H, $CH_3$ of $C_2H_5$, $J_{1,2}$ = 7.6Hz), 2.71 (q, 2H, $CH_2$ of $C_2H_5$), 4.71 (s, 2H, $NH_2$), 5.25 (s, 2H, $OCH_2$), 6.58 (s, 1H, $C_3$-H), 6.62 (t, 1H, $C_{17}$-H, $J_{1,2}$ = 4.8 Hz), 7.00 (d, 1H ,$C_{18}$-H, $J_{1,2}$ = 6.8 Hz), 7.31 (s, 1H, $C_5$-H), 7.34 (d, 1H, $C_8$-H, $J_{1,2}$ = 4.4 Hz), 7.41 (d, 1H, $C_7$-H, $J_{1,2}$ = 6.4 Hz), 7.75 (d, 1H, $C_{16}$-H, $J_{1,2}$ = 6 Hz) ppm; Anal. Calcd for $C_{17}H_{16}N_2O_3$: C, 68.91: H, 5.44: N, 9.45. Found: C, 68.88: H, 5.21: N, 9.21.

#### 2.2.5. 6-Isopropyl-4-((2-amino-pyridin-3-yloxy) methyl)-2*H*-chromen-2-one (3e)

Yield 91%; colorless solid; m.p.: 194-196 °C; IR (KBr, cm$^{-1}$): 1710 cm$^{-1}$ (lactone C = O), 3137 cm$^{-1}$, 3058 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 1.27 (d, 6H, 2-$CH_3$ of isopropyl, $J_{1,2}$ = 6.8Hz), 2.95 (m, 1H, CH of isopropyl, $J_{1,2}$ = 7.2Hz), 4.71 (s, 2H, $NH_2$, $D_2O$ exchangeable), 5.26 (s, 2H, $OCH_2$), 6.58 (s, 1H, $C_3$-H), 6.63 (t, 1H, $C_{17}$-H, $J_{1,2}$ = 5.2 Hz), 7.01

(d, 1H, $C_{18}$-H, $J_{1,2}$ = 7.2 Hz), 7.33-7.35 (m, 2H, $C_5$-H & $C_8$-H), 7.45 (d, 1H, $C_7$-H, $J_{1,2}$ = 6.8 Hz), 7.75 (d, 1H, $C_{16}$-H, $J_{1,2}$ = 6 Hz) ppm; $^{13}$C NMR (400 MHz, $CDCl_3$): $\delta$ = 24.55 ($C_{21}$ and $C_{20}$), 34.31 ($C_{19}$), 66.42 ($C_{11}$), 114.07 ($C_3$), 114.11 ($C_{10}$), 117.26($C_{18}$), 117.92 ($C_8$), 121.14 ($C_{17}$), 131.17 ($C_7$), 140.89 ($C_5$), 140.93 ($C_{16}$), 145.86 ($C_6$), 149.71 ($C_9$), 150.56 ($C_{13}$ and $C_{14}$), 152.50 ($C_4$), 161.08 ($C_2$) ppm; LC-MS: *m/z* = 311(M+H); Anal. Calcd for $C_{18}H_{18}N_2O_3$: C, 69.66: H, 5.85: N, 9.03. Found: C, 69.31: H, 5.60: N, 8.79.

### 2.2.6. 6-*tert*-Butyl-4-((2-amino-pyridin-3-yloxy) methyl)-2*H*-chromen-2-one (3f)

Yield 91%; colorless solid; m.p.: 184-186 °C; IR (KBr): 1697 cm$^{-1}$ (lactone C = O), 3143 cm$^{-1}$, 3089 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 1.35 (s, 9H, 6-*tert*-butyl), 4.70 (s, 2H, $NH_2$), 5.27 (s, 2H, $OCH_2$), 6.58 (s, 1H, $C_3$-H), 6.63 (t, 1H, $C_{17}$-H, $J_{1,2}$ = 4.8 Hz), 7.01 (d, 1H, $C_{18}$-H, $J_{1,2}$ = 6.8 Hz), 7.33 (d, 1H, $C_8$-H, $J_{1,2}$ = 8.8 Hz), 7.48 (s, 1H, $C_5$-H), 7.63 (d, 1H, $C_7$-H, $J_{1,2}$ = 6.8 Hz), 7.75 (d, 1H, $C_{16}$-H, $J_{1,2}$ = 6.4 Hz) ppm); Anal. Calcd for $C_{19}H_{20}N_2O_3$: C, 70.35: H, 6.21: N, 8.64. Found: C, 70.11: H, 6.03: N, 8.32.

### 2.2.7. 6-Benzyl-4-((2-amino-pyridin-3-yloxy) methyl)-2*H*-chromen-2-one (3g)

Yield 95%; colorless solid; m.p.: 173-175 °C; IR (KBr): 1701 cm$^{-1}$ (lactone C = O), 3338 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 4.04 (s, 2H, $C_6$-$CH_2$), 4.63 (s, 2H, $NH_2$), 5.19 (s, 2H, $OCH_2$), 6.57 (s, 1H, $C_3$-H), 6.62 (t, 1H, $C_{17}$-H, $J_{1,2}$ = 4.8 Hz), 6.96-7.76 (m, 10H, Ar-H) ppm; Anal. Calcd for $C_{22}H_{18}N_2O_3$: C, 73.73: H, 5.06: N, 7.82. Found: C, 73.21: H, 5.01: N, 7.62.

### 2.2.8. 1-((2-Amino-pyridin-3-yloxy) methyl)-3H- benzo(*f*)chromen-3-one (3h)

Yield 94%; colorless solid; m.p.: 228-230 °C; IR (KBr): 1718 cm$^{-1}$ (lactone C = O), 3153 cm$^{-1}$, 3074 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ = 5.76 (s, 2H, $OCH_2$), 5.80 (s, 2H, $NH_2$), 6.53 (t, 1H, $C_{21}$-H, $J_{1,2}$ = 4.8 Hz), 6.95 (s, 1H, $C_3$-H), 7.28-8.33 (m, 8H, Ar-H) ppm; Anal. Calcd for $C_{19}H_{14}N_2O_3$: C, 71.69: H, 4.43: N, 8.80. Found: C, 71.21: H, 4.34: N, 8.62.

### 2.2.9. 6-Chloro-4-((2-amino-pyridin-3-yloxy) methyl)-2*H*-chromen-2-one (3i)

Yield 92%; colorless solid; m.p.: 233-235 °C; IR (KBr): 1699 cm$^{-1}$ (lactone C = O), 3105 cm$^{-1}$, 3064 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ = 5.36 (s, 2H, $OCH_2$), 5.92 (s, 2H, $NH_2$), 6.50 (t, 1H, $C_{17}$-H, $J_{1,2}$ = 5.2 Hz), 7.0 (s, 1H, $C_3$-H), 7.37 (d, 1H, $C_8$-H, $J_{1,2}$ = 7.2 Hz), 7.47 (d, 1H, $C_{18}$-H, $J_{1,2}$ = 8.8 Hz), 7.55 (d, 1H, $C_{16}$-H, $J_{1,2}$ = 4.4 Hz), 7.67 (d, 1H, $C_7$-H, $J_{1,2}$ = 4.4 Hz), 8.01 (s, 1H, $C_5$-H) ppm; Anal. Calcd for $C_{15}H_{11}ClN_2O_3$: C, 59.52: H, 3.66: N, 9.25. Found: C, 59.21: H, 3.34: N, 9.09.

### 2.2.10. 6-Bromo-4-((2-amino-pyridin-3-yloxy) methyl)-2*H*-chromen-2-one (3j)

Yield 89%; colorless solid; m.p.: 231-233 °C; IR (KBr): 1708 cm$^{-1}$ (lactone C = O), 3105 cm$^{-1}$, 3060 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ = 5.36 (s, 2H, $OCH_2$), 5.92 (s, 2H, $NH_2$), 6.50 (t, 1H, $C_{17}$-H, $J_{1,2}$ = 4.8 Hz), 6.99 (s, 1H, $C_3$-H), 7.38-7.40 (m, 2H, $C_{18}$-H and $C_8$-H), 7.55 (d, 1H, $C_{16}$-H, $J_{1,2}$ = 4.8 Hz), 7.78 (d, 1H, $C_7$-H, $J_{1,2}$ = 6.8 Hz), 8.12 (s, 1H, $C_5$-H) ppm; Anal. Calcd for $C_{15}H_{11}BrN_2O_3$: C, 51.90: H, 3.19: N, 8.07. Found: C, 51.21: H, 3.11: N, 8.01.

### 2.2.11. 6-Fluoro-4-((2-amino-pyridin-3-yloxy) methyl)-2*H*-chromen-2-one (3k)

Yield 88%; colorless solid; m.p.: 230-232 °C; IR (KBr): 1716 cm$^{-1}$ (lactone C = O), 3141 cm$^{-1}$, 3070 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$): δ = 5.34 (s, 2H, $OCH_2$), 5.92 (s, 2H, $NH_2$), 6.51 (t, 1H, $C_{17}$-H, $J_{1,2}$ = 5.2 Hz), 7.00 (s, 1H, $C_3$-H), 7.35 (d, 1H, $C_{17}$-H, $J_{1,2}$ = 6.8 Hz), 7.49-7.55 (m, 3H, $C_5$-H, $C_8$-H, and $C_{16}$-H), 7.79 (d, 1H, $C_7$-H, $J_{1,2}$ = 6.8 Hz) ppm; Anal. Calcd for $C_{15}H_{11}FN_2O_3$: C, 62.94: H, 3.87: N, 9.79. Found: C, 62.69: H, 3.78: N, 9.71.

### 2.2.12. 7-Methyl-4-((2-methyl-pyridin-3-yloxy) methyl)-2*H*-chromen-2-one (3l)

Yield 86%; colorless solid; m.p.: 220-222 °C; IR (KBr): 1701 cm$^{-1}$ (lactone C = O); $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ = 2.42 (s, 3H, $C_7$-$CH_3$), 2.49 (s, 3H, $C_{14}$-$CH_3$), 5.44 (s, 2H, $OCH_2$), 6.50 (s, 1H, $C_3$-H), 7.21-7.28 (m, 3H, $C_{17}$-H, $C_{18}$-H, and $C_8$-H), 7.56 (d, 1H, $C_6$-H, $J_{1,2}$ = 7.2 Hz), 7.76 (d, 1H, $C_5$-H, $J_{1,2}$ = 8 Hz), 8.05 (d, 1H, $C_{16}$-H, $J_{1,2}$ = 5.6 Hz) ppm; Anal. Calcd for $C_{17}H_{15}NO_3$: C, 72.58: H, 5.37: N, 4.98. Found: C, 72.22: H, 5.12: N, 4.60.

### 2.2.13. 6-Isopropyl-4-((2-methyl-pyridin-3-yloxy) methyl)-2*H*-chromen-2-one (3m)

Yield 89%; colorless solid; m.p.: 160-162 °C; IR (KBr): 1704 cm$^{-1}$ (lactone C = O); $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ = 1.24 (d, 6H, 2-$CH_3$ of isopropyl, $J_{1,2}$ = 7.2 Hz), 2.50 (s, 3H, $C_{14}$-$CH_3$), 3.01 (m, 1H, CH of isopropyl, $J_{1,2}$ = 7.2 Hz), 5.49 (s, 2H, $OCH_2$), 6.57 (s, 1H, $C_3$-H), 7.25 (t, 1H, $C_{17}$-H, $J_{1,2}$ = 4.8 Hz), 7.38 (d, 1H, $C_{18}$-H, $J_{1,2}$ = 8.8 Hz), 7.56 (d, 1H, $C_7$-H, $J_{1,2}$ = 6.4 Hz), 7.63 (d, 1H, $C_8$-H, $J_{1,2}$ = 7.6 Hz), 7.71 (s, 1H, $C_5$-H), 8.07 (d, 1H, $C_{16}$-H, $J_{1,2}$ = 6 Hz) ppm; $^{13}$C NMR (400 MHz, $CDCl_3$): $\delta$ = 20.09 ($C_{22}$), 24.55 ($C_{21}$ and $C_{20}$), 34.34 ($C_{19}$), 65.99 ($C_{11}$), 113.93 ($C_3$), 117.24 ($C_{18}$), 117.90 ($C_{10}$), 118.28 ($C_8$), 121.09 ($C_{17}$), 122.23 ($C_5$), 131.12 ($C_7$), 142.21 ($C_{16}$), 145.80 ($C_6$), 149.68 ($C_9$), 149.85 ($C_{13}$ and $C_{14}$), 152.45 ($C_4$), 161.16 ($C_2$) ppm; Anal. Calcd for $C_{19}H_{19}NO_3$: C, 73.77: H, 6.19: N, 4.53. Found: C, 73.52: H, 6.02: N, 4.44.

### 2.2.14. 6-*tert*-Butyl-4-((2-methyl-pyridin-3-yloxy) methyl)-2*H*-chromen-2-one (3n)

Yield 90%; colorless solid; m.p.: 136-137 °C; IR (KBr): 1706 cm$^{-1}$ (lactone C = O); $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 1.36 (s, 9H, 6-*tert*-butyl), 2.56 (s, 3H, $C_{14}$-$CH_3$), 5.28 (s, 2H, $OCH_2$), 6.64 (s,

1H, $C_3$-H), 7.13-7.19 (m, 2H, $C_{17}$-H and $C_{18}$-H), 7.33 (d, 1H, $C_8$-H, $J_{1,2}$ = 8.8 Hz), 7.50 (s, 1H, $C_5$-H), 7.62 (d, 1H, $C_7$-H, $J_{1,2}$ = 6.4 Hz), 8.17 (d, 1H, $C_{16}$-H, $J_{1,2}$ = 5.6 Hz) ppm; Anal. Calcd for $C_{20}H_{21}NO_3$: C, 74.28: H, 6.55: N, 4.33. Found: C, 74.11: H, 6.43: N, 4.23.

**2.2.15. 1-((2-Methyl-pyridin-3-yloxy) methyl)-3H- benzo (*f*) chromen-3-one (3o)**

Yield 89%; colorless solid; m.p.: 222-224 °C; IR (KBr): 1706 $cm^{-1}$ (lactone C = O); $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ = 2.38 (s, 3H, $C_{18}$-$CH_3$), 5.83 (s, 2H, $OCH_2$), 6.81 (s, 1H, $C_3$-H), 7.24 (t, 1H, $C_{21}$-H, $J_{1,2}$ = 4.8 Hz), 7.60-8.32 (m, 8H, Ar-H) ppm; Anal. Calcd for $C_{20}H_{15}NO_3$: C, 75.70: H, 4.76: N, 4.41. Found: C, 75.31: H, 4.43: N, 4.23.

**2.2.16. 6-Chloro-4-((2-methyl-pyridin-3-yloxy) methyl)-2*H*-chromen-2-one (3p)**

Yield 94%; colorless solid; m.p.: 226-228 °C; IR (KBr): 1706 $cm^{-1}$ (lactone C = O); $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ = 2.46 (s, 3H, $C_{14}$-$CH_3$), 5.46 (s, 2H, $OCH_2$), 6.62 (s, 1H, $C_3$-H), 7.23 (t, 1H, $C_{17}$-H, $J_{1,2}$ = 4.8 Hz), 7.49 (d, 1H, $C_{18}$-H, $J_{1,2}$ = 8.8 Hz), 7.63 (d, 1H, $C_8$-H, $J_{1,2}$ = 7.6 Hz), 7.70 (d, 1H, $C_7$-H, $J_{1,2}$ = 6.4 Hz), 8.01 (s, 1H, $C_5$-H), 8.06 (d, 1H, $C_{16}$-H, $J_{1,2}$ = 4.8 Hz) ppm; Anal. Calcd for $C_{16}H_{12}ClNO_3$: C, 63.69: H, 4.01: N, 4.64. Found: C, 63.21: H, 3.98: N, 4.39.

**2.2.17. 6-Bromo-4-((2-methyl-pyridin-3-yloxy) methyl)-2*H*-chromen-2-one (3q)**

Yield 93%; colorless solid; m.p.: 234-236 °C; IR (KBr): 1710 $cm^{-1}$ (lactone C = O); $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ = 2.46 (s, 3H, $C_{14}$-$CH_3$), 5.46 (s, 2H, $OCH_2$), 6.61 (s, 1H, $C_3$-H), 7.22 (t, 1H, $C_{17}$-H, $J_{1,2}$ = 4.8 Hz), 7.42 (d, 1H, $C_{18}$-H, $J_{1,2}$ = 8.8 Hz), 7.63 (d, 1H, $C_8$-H, $J_{1,2}$ = 7.6 Hz), 7.81 (d, 1H, $C_7$-H, $J_{1,2}$ = 6.4 Hz), 8.06 (d, 1H, $C_{16}$-H, $J_{1,2}$ = 4.8 Hz), 8.13 (s, 1H, $C_5$-H) ppm; Anal. Calcd for $C_{16}H_{12}BrNO_3$: C, 55.51: H, 3.49: N, 4.05. Found: C, 55.21: H, 3.33: N, 4.01.

**2.2.18. 6-Fluoro-4-((2-methyl-pyridin-3-yloxy) methyl)-2*H*-chromen-2-one (3r)**

Yield 95%; colorless solid; m.p.:233-235 °C; IR (KBr); 1701 $cm^{-1}$ (lactone C = O); $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ = 2.46 (s, 3H, $C_{14}$-$CH_3$), 5.43 (s, 2H, $OCH_2$), 6.62 (s, 1H, $C_3$-H), 7.23 (t, 1H, $C_{17}$-H, $J_{1,2}$ = 4.8 Hz), 7.51-7.56 (m, 2H, $C_{18}$-H and $C_5$-H), 7.60 (d, 1H, $C_8$-H, $J_{1,2}$ = 7.6 Hz), 7.77 (d, 1H, $C_7$-H, $J_{1,2}$ = 6.8 Hz), 8.06 (d, 1H, $C_{16}$-H, $J_{1,2}$ = 5.6 Hz) ppm; Anal. Calcd for $C_{16}H_{12}FNO_3$: C, 67.37: H, 4.24: N, 4.91. Found: C, 67.21: H, 4.11: N, 4.87.

**3. RESULTS AND DISCUSSION**

The structures of novel pyridinoxymethylcoumarins were established from IR, $^1H$ NMR, $^{13}C$ NMR and LC-MS data as illustrated for a representative example. In the IR spectrum of 6-isopropyl-4-((2-amino-pyridin-3-yloxy)methyl)-*2H*-chromen-2-one (3e) [$R^1$ = 6-*i*-Pr, $R^2$ = $NH_2$], the lactone carbonyl stretching frequency was appeared at 1710 $cm^{-1}$, where as $NH_2$ stretching frequency was appeared at 3137 $cm^{-1}$ and 3058 $cm^{-1}$. The $^1H$ NMR spectrum of the compound (3e) displayed a singlet at δ 4.71, 5.26 and 6.58 due to $NH_2$ ($D_2O$ exchangeable), $OCH_2$ and $C_3$-H respectively. A multiplet was observed at $\delta$ 2.95 ($J_{1,2}$ = 7.2 Hz) due to methine proton of isopropyl group. The two methyl group of isopropyl, $C_{18}$-H, $C_7$-H and $C_{16}$-H protons were resonated as a doublet at δ 1.27 ($J_{1,2}$ = 6.8 Hz), 7.01 ($J_{1,2}$ = 7.2 Hz), 7.45 ($J_{1,2}$ = 6.8 Hz), and 7.75 ($J_{1,2}$ = 6 Hz) respectively. The $C_5$-H and $C_8$-H protons appeared as a multiplet in the range of δ 7.33-7.35 and $C_{17}$-H proton as a triplet at $\delta$ 6.63 ($J_{1,2}$ = 5.2 Hz).

The mass spectrum (LC-MS) of the compound (3e) exhibited a [M+H] peak at 311, which is in agreement with the molecular formula $C_{18}H_{18}N_2O_3$. The $^{13}C$ NMR spectral data of compound (3e) ($R^1$ = 6-*iso*-Pr, $R^2$ = $NH_2$) and (3m) ($R^1$ = 6-*iso*-Pr, $R^2$ = $CH_3$) are given in experimental section, which are confirmed by their HSQC spectrum.

**3.1. Biological activity**

**3.1. 2. *In vitro* cytotoxic screening**

The newly synthesized compounds were evaluated for their *in vitro* cytotoxic activity against Dalton's Ascites Lymphoma (DAL) and Ehrlich Ascites Carcinoma (EAC) cells. *5-Fluorouracil* was used as the reference drug in this study.

The relationship between surviving fraction and drug concentration was plotted to obtain the survival curve of Dalton's Ascites Lymphoma (DAL) and Ehrlich Ascites Carcinoma (EAC) cells. The response parameter calculated was the $IC_{50}$ value, which corresponds to the concentration required for 50% inhibition of cell viability.

The $IC_{50}$ of the synthesized compounds compared to the reference drug are shown in Table 1 and the results are represented graphically in Figure 2.

The investigation of *in vitro* cell cytotoxicity against DAL cell revealed that the most of the tested compounds exhibited good activity. The compound (3o) ($R^1$ = 5,6-Benzo, $R^2$ = $CH_3$) was the most potent cytotoxic compound in this screening against DAL cell with $IC_{50}$ value of 30.21µg/mL. The compounds (3b) ($R^1$ = 7-$CH_3$, $R^2$ = $NH_2$), (3m) ($R^1$ = 6-*i*-Pr, $R^2$ = $CH_3$), (3p) ($R^1$ = 6-Cl,

$R^2$ = $CH_3$), (3q) ($R^1$ = 6-Br, $R^2$ = $CH_3$) and (3r) ($R^1$ = 6-F, $R^2$ = $CH_3$) were found to be highly

**Table - 1: Results of *in vitro* cytotoxic activity of the synthesized compounds on DAL and EAC cells**

| Compounds | $R_1$ | $R_2$ | DAL $IC_{50}$ | EAC $IC_{50}$ |
|---|---|---|---|---|
| **3a** | 6-$CH_3$ | $NH_2$ | 86.41 | 56.26 |
| **3b** | 7-$CH_3$ | $NH_2$ | 68.74 | 51.20 |
| **3c** | 6-$OCH_3$ | $NH_2$ | 86.79 | 148.24 |
| **3d** | 6-Et | $NH_2$ | 79.23 | 103.42 |
| **3e** | 6-*i*-Pr | $NH_2$ | 80.20 | 155.24 |
| **3f** | 6-*tert*-Butyl | $NH_2$ | 93.35 | 79.21 |
| **3g** | 6-Benzyl | $NH_2$ | 94.60 | 87.24 |
| **3h** | 5,6-Benzo | $NH_2$ | 85.22 | 135.21 |
| **3i** | 6-Cl | $NH_2$ | 104.67 | 95.25 |
| **3j** | 6-Br | $NH_2$ | 106.37 | 125.30 |
| **3k** | 6-F | $NH_2$ | 78.24 | 109.27 |
| **3l** | 7-$CH_3$ | $CH_3$ | 86.07 | 57.21 |
| **3m** | 6-*i*-Pr | $CH_3$ | 75.81 | 37.34 |
| **3n** | 6-*tert*-Butyl | $CH_3$ | 87.51 | 99.12 |
| **3o** | 5,6-Benzo | $CH_3$ | 30.21 | 150.38 |
| **3p** | 6-Cl | $CH_3$ | 75.14 | 115.34 |
| **3q** | 6-Br | $CH_3$ | 47.21 | 120.42 |
| **3r** | 6-F | $CH_3$ | 64.97 | 117.28 |
| ***5-Fluorouracil*** | | | 41.6 | 41.6 |

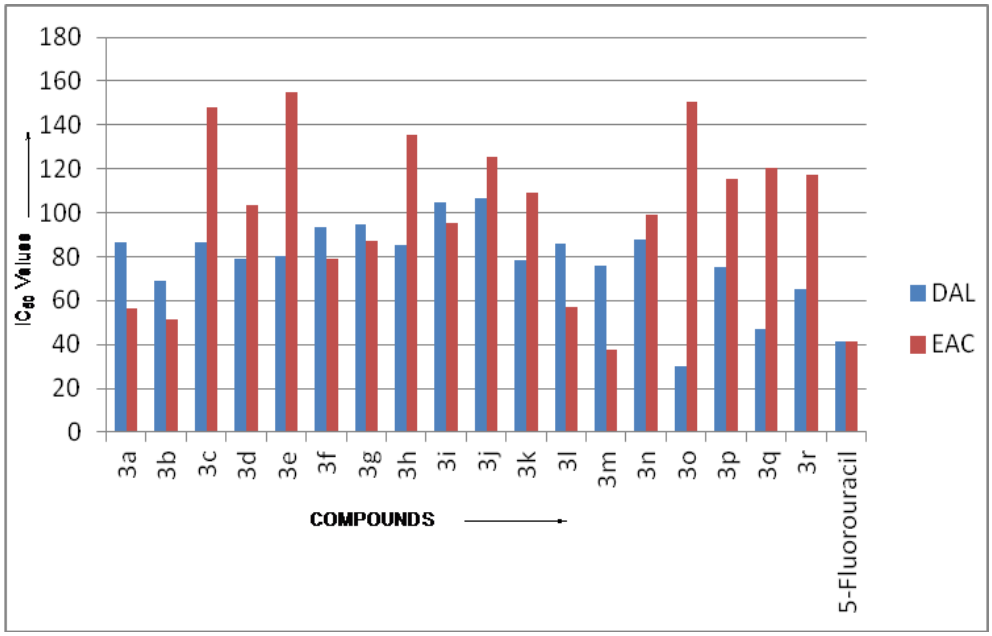



**Figure - 2: $IC_{50}$ of the synthesized compounds compared to the reference drug**

active against DAL cells with $IC_{50}$ value between 47.21 and 75.81µg/mL. The compounds (3a) ($R^1$ = 6-$CH_3$, $R^2$ = $NH_2$), (3c) ($R^1$ = 6-$OCH_3$, $R^2$ = $NH_2$), (3d) ($R^1$ = 6-Et, $R^2$ = $NH_2$), (3e) ($R^1$ = 6-*i*-Pr, $R^2$ = $NH_2$), (3h) ($R^1$ = 5,6-Benzo, $R^2$ = $NH_2$), (3k) ($R^1$ = 6-F, $R^2$ = $NH_2$), (3l) ($R^1$ = 7-$CH_3$, $R^2$ = $CH_3$) and (3n) ($R^1$ = 6-

*tert*-Butyl, $R^2 = CH_3$) showed moderate activity against DAL cell with $IC_{50}$ value between 79.23 and 87.51µg/mL. The compounds (3f) ($R^1$ = 6-*tert*-Butyl, $R^2 = NH_2$), (3g) ($R^1$ = 6-Benzyl, $R^2 = NH_2$), (3i) ($R^1$ = 6-Cl, $R^2 = NH_2$) and (3j) ($R^1$ = 6-Br, $R^2 = NH_2$) showed poor activity against DAL cell with $IC_{50}$ value between 93.35 and 106.37µg/mL.

The investigation of *in vitro* cell cytotoxicity against EAC cell revealed that most of the tested compounds exhibited good activity. The compound (3m) ($R^1$ = 6-*i*-Pr, $R^2 = CH_3$) was the most potent cytotoxic compound in this screening against EAC cell with $IC_{50}$ value of 37.34µg/mL. The compounds (3a) ($R^1 = 6\text{-}CH_3$, $R^2 = NH_2$), (3b) ($R^1 = 7\text{-}CH_3$, $R^2 = NH_2$), (3f) ($R^1$ = 6-*tert*-Butyl, $R^2 = NH_2$) and (3l) ($R^1 = 7\text{-}CH_3$, $R^2 = CH_3$) were found to be highly active against EAC cell with $IC_{50}$ value between 51.20 and 79.21µg/mL. The compound (3g) ($R^1$=6-Benzyl, $R^2$=$NH_2$) showed moderate activity against EAC cell with $IC_{50}$ value of 87.24µg/mL. The compounds (3c) ($R^1 = 6\text{-}OCH_3$, $R^2 = NH_2$), (3d) ($R^1$ = 6-Et, $R^2 = NH_2$), (3e) ($R^1$ = 6-*i*-Pr, $R^2 = NH_2$), (3h) ($R^1$ = 5,6-Benzo, $R^2 = NH_2$), (3i) ($R^1$ = 6-Cl, $R^2 = NH_2$), (3j) ($R^1$ = 6-Br, $R^2 = NH_2$), (3k) ($R^1$ = 6-F, $R^2 = NH_2$), (3n) ($R^1$ = 6-*tert*-Butyl, $R^2 = CH_3$), (3o) ($R^1$ = 5,6-Benzo, $R^2 = CH_3$), (3p) ($R^1$ = 6-Cl, $R^2 = CH_3$), (3q) ($R^1$ = 6-Br, $R^2 = CH_3$) and (3r) ($R^1$ = 6-F, $R^2 = CH_3$) showed poor activity against EAC cell with $IC_{50}$ between 95.25 and 155.24µg/mL.

## 4. CONCLUSION

Introduction of 5,6-benzo group in the coumarin ring is found enhance the *in vitro* cytotoxic activity against DAL cell line. Similarly, introduction of isopropyl group at 6-position in the coumarin ring is found enhance the *in vitro* cytotoxic activity against EAC cell line.

***Acknowledgment***

One of the authors (N.J) gratefully acknowledges the financial support from the Council Scientific and Industrial Research, New Delhi, in the form of Junior Research Fellowship [Grant No. 20-06/2010(i) EU-IV]. We are thankful to Indian Institute of Science, Bangalore for the spectral analysis.

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