Research Article

Synthesis and Cytotoxic Studies of A New Series of Quinolinoxymethylcoumarins

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ABSTRACT

4-Bromomethylcoumarins (1a-k) were reacted with 8-hydroxyquinolines (2a-b) to yield quinolinoxymethylcoumarins (3a-o). The structure of all the synthesized compounds were confirmed by spectral studies and screened for their anticancer activities against Dalton's Ascitic Lymphoma (DAL) and Ehrlich Ascites Carcinoma (EAC) cell lines. Out of these, the compound (3d) (R = 6-Benzyl, R_1=H) was found to be the most potent cytotoxic compound against DAL cell line with IC_{50} value of 45.86 µg/mL and the compound (3i) (R = 6-i-Pr, R_1= CH_3) against EAC cell line with IC_{50} value of 39.26 µg/mL.

Keywords: 4-Bromomethylcoumarins, Coumarins, Cytotoxic activity, Quinoline.

INTRODUCTION

Many quinoline nucleus containing compounds exhibited a wide variety of pharmacological and biological activities. 2-Chloro-8-methylquinolineamine derivatives[1] emerged as most potential antidepressant agents by forced swim test in rats. Bisquinolines[2] synthesized from 8-hydroxyquinolines possessed significant antibacterial activity against *Escherichia coli.* Quinoline based thiazolidinones[3] were found to be the most active antifungal agents against *Candida albicans.* β-Aryloxyquinoline derivatives[4] were emerged as the promising antitubercular member against *Mycobacterium tuberculosis* H37Rv. 3-Arylquinolines[5] were readily synthesized by a novel Friedlander-type reaction and explored with the proofs of isolation of the enaminone intermediate. 8-Hydroxyquinoline[6] is a versatile ligand in coordination chemistry which was used for analytical purposes.

Coumarins are known to be biologically versatile compounds possessing several biological properties. Thiazolidinyl-4-aryloxymethylcoumarins[7] were found to be potent anti-inflammatory agents by carrageenan induced rat paw oedema inhibition method and numerous research reports[8] have also indicated the coumarin nucleus as a potential candidate for development of anti inflammatory drugs. Halogenated-4-aryloxymethylcoumarins[9] was found to be potent antibacterial agents against *Bacillus subtils* and *Escherichia coli*. 3,4-Dihydropyridin-2-one-4-aryloxy methylcoumarins[10] were also found to be potent antifungal agents against *Penicillium chrysogenum* and *Rhizopus oryzae.* 4-Amino-3-(2-methylbenzyl) coumarin derivatives[11] showed potent estrogenic activity on the estrogen receptor positive (ER^+) human MCF-7 breast cancer cell line. Benzothiazolyl coumarin acetamide derivatives[12] possessed strong *in vitro* anti-HIV effect against the wild-type HIV-1 cell line. Pyrrole bis coumarins, a new structure for florescent probes has been reported.[13] A protocol for chemoselective one pot synthesis of Benzthiazinyl coumarins has been developed.[14]

On the basis of all of these evidences, we set out to synthesize a new series of biologically active compounds containing both of these two important pharmacophores. This study presents the synthesis, characterization and *in vitro* cytotoxic activities of these new quinolinoxy methylcoumarins.

Chemistry

Various 4-bromomethylcoumarins (1a-k)[15,16] were synthesized by the Pechmann cyclisation of phenols with 4-bromoethylacetoacetate.[17] In the same method, we have synthesized a novel 4-bromomethyl-6-ethyl coumarin (1d) (scheme 1).

4-Bromomethyl-6-methylcoumarins (1a-k) reacted with 8-hydroxyquinolines (2a-b) in the presence of anhydrous K_2CO_3 to give 6-methyl-4-(quinolin-8-yloxymethyl)-chromone-2-one.[18] We have extended the same method to other substituent of 4-bromomethylcoumarins **(1a-k)** and 8-hydroxyquinolines (2a-b) (Scheme 2). The numbering of the skeleton (3e) is shown (Figure 1).

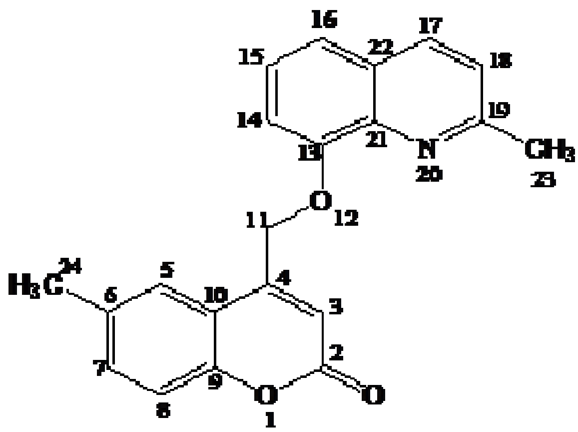
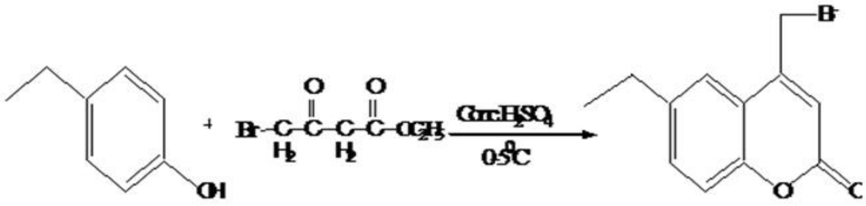


Figure 1: (3e)

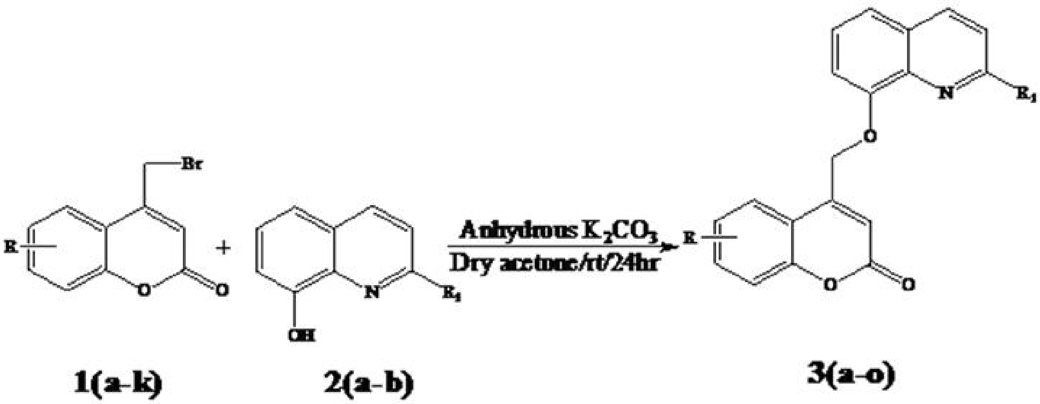
MATERIALS AND METHODS

General

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 400 MHz spectrometer by using $CDCl_3$ as a solvent and TMS as an internal standard. The chemical shifts are expressed in δ ppm. The mass spectra were recorded using Agilent-Single Quartz LC-MS. The purity of the compound was checked by TLC.



Scheme 1: Synthesis of 4-Bromomethyl-6-ethylcoumarin **(1d)**



Scheme 2: Synthesis of quinolinoxymethylcoumarins 3(a=0)

R = 1a; 6-CH_3, 1b; 7-CH_3, 1c; 6-OMe, 1d; 6-C_2H_5, 1e; 6-ipr, 1f; 6-tert-Butyl, 1g; Benzyl, 1h; 5,6-Benzo, 1i; 6-Cl, 1j; 6-Br, 1k; 6-F.

R_1 = 2a; H, 2b; CH_3.

General procedure

Synthesis of 4-bromomethyl-6-ethylcoumarin (1d)

To a mixture of equimolar quantity of 4-ethylphenol (5.0 g, 40.7 mmol) and 4-bromoethylacetoacetate (8.5 mL, 40.7 mmol) was added drop wise Conc. sulphuric acid (5mL) with stirring and maintaining the temperature between 0-5°C. The reaction mixture was allowed to stand in ice chest overnight and deep red colored solution was poured into the stream of crushed ice. Solid separated was filtered, washed with water, dried, and recrystallized from acetic acid.

Yield 95%; colorless solid; m.p. 228 ^{0}C; IR (KBr, cm^{-1}) 1701 cm^{-1} (lactone C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.26 (t, 3H, CH_3 of C_2H_5, $J_{1,2}$ = 8 Hz), 2.71 (q, 2H, CH_2 of C_2H_5, $J_{1,2}$ = 7.6 Hz), 4.50 (s, 2H, CH_2-Br), 6.51 (s, 1H, C_3-H), 7.26 (d, 1H, C_8-H, $J_{1,2}$ = 5.2 Hz), 7.39 (d, 1H, C_7-H, $J_{1,2}$ = 10.4 Hz), 7.50 (s, 1H, C_5-H).

Synthesis of 4-(quinolin-8-yloxymethyl)-chromone-2-one (3a-o)

One of the 8-hydroxyquinolines (**2a-b**) (3.4 mmol) and anhydrous K_2CO_3 (1.38g, 10mmol) were stirred in 25 ml of dry acetone for 30 min. One of the 4-bromomethylcoumarins (**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 10 ml of 5% HCl. Then, it was washed with 50 ml of cold water. The crude product was dried and recrystallized from ethanol.

6-Ethyl-4-(quinolin-8-yloxymethyl)-chromen-2-one ***(3a)***

Yield 95%; colorless solid; m.p. 204 - 206 ^{0}C; IR (KBr, cm^{-1}) 1721 cm^{-1} (lactone C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25 (t, 3H, CH_3 of C_2H_5), 2.70 (q, 2H, CH_2 of C_2H_5, $J_{1,2}$ = 7.6 Hz), 5.64 (S, 2H, OCH_2), 6.70(s, 1H, C_3-H), 7.05 (d, 1H, C_{16}-H, $J_{1,2}$ = 8.4 Hz), 7.30 (d, 1H , C_{14}-H, $J_{1,2}$ = 8.4 Hz), 7.40 -7.53 (m, 5H, C_5, C_7, C_8, C_{15} & C_{18}-H), 8.20 (d, 1H, C_{17}-H, $J_{1,2}$ = 7.2 Hz), 9.0 (d, 1H, C_{19}-H, $J_{1,2}$ = 5.6 Hz).

6-Isopropyl-4-(quinolin-8-yloxymethyl)-chromen-2-one ***(3b)***

Yield 85%; colorless solid; m.p. 185 - 187 ^{0}C; IR (KBr, cm^{-1}) 1723 cm^{-1} (lactone C=O), ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.26 (d, 6H, 2-CH_3 of isopropyl, $J_{1,2}$ =6 Hz), 2.9 (septd, 1H, CH of isopropyl, $J_{1,2}$ = 3 Hz), 5.62 (s, 2H, OCH_2), 6.70 (s, 1H, C_3-H), 7.04 (d, 1H, C_{16}-H, $J_{1,2}$ = 8.8 Hz), 7.30 (d, 1H , C_{14}-H, $J_{1,2}$ = 8.8 Hz), 7.39 – 7.43 (m, 2H, C_5 & C_{15}-H, $J_{1,2}$ = 10 Hz), 7.46 - 7.50 (m, 3H, C_7, C_8 & C_{17}-H, $J_{1,2}$ = 2 Hz), 8.15 (t, 1H, C_{18}-H, $J_{1,2}$ = 8.4 Hz), 8.96 (d, 1H, C_{19}-H, $J_{1,2}$ = 4 Hz).

*6-Tert-butyl-4-(quinolin-8-yloxymethyl)-chromen-2-one **(3c)***

Yield 85%; brown solid; m.p. 139 - 141 ^{0}C; IR (KBr, cm^{-1}) 1690cm^{-1} (lactone C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.35 (s, 9H, 6-tert-butyl), 5.66 (s, 2H, OCH_2), 6.69 (s, 1H, C_3-H), 7.07 (d, 1H, C_{14}-H, $J_{1,2}$ = 7.2 Hz), 7.32 (d, 1H , C_8-H, $J_{1,2}$ = 8.8 Hz), 7.44 (t, 1H, C_{15}-H, $J_{1,2}$ = 8 Hz), 7.51-7.53 (m, 2H, C_5, C_{18}-H), 7.64 (d, 1H, C_{16}-H, $J_{1,2}$ = 10.8 Hz), 7.66 (d ,1H, C_7-H, $J_{1,2}$ = 2 Hz), 8.21 (d, 1H, C_{17}-H, $J_{1,2}$ = 8.4 Hz), 9.0 (d, 1H, C_{19}-H, $J_{1,2}$ = 4 Hz).

*6-Benzyl-4-(quinolin-8-yloxymethyl)-chromen-2-one **(3d)***

Yield 95%; yellow solid; m.p. 204 - 206 ^{0}C; IR (KBr, cm^{-1}) 1735 cm^{-1} (lactone C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.06 (s, 2H, C_6-CH_2), 5.58 (s, 2H, OCH_2), 6.71 (s, 1H, C_3-H), 7.03-8.99 (m, 14H, Ar-H).

*6-Methyl-4-(2-methyl-quinolin-8-yloxymethyl)-chromen-2-one **(3e)***

Yield 95%; colorless solid; m.p. 219 - 221 ^{0}C; IR (KBr, cm^{-1}) 1716 cm^{-1} (lactone C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.38 (s, 3H, 6-CH_3), 2.75 (s, 3H, C_{19}-CH_3), 5.55 (s, 2H, OCH_2), 6.68 (s, 1H, C_3-H), 6.94 (d, 1H, C_7-H, $J_{1,2}$ = 7.6 Hz), 7.19 (d, 1H, C_8-H, $J_{1,2}$ = 6.4 Hz), 7.23 (d, 1H, C_{14}-H, $J_{1,2}$ = 6.8 Hz), 7.26-7.32 (m, 2H, $C_{15,}$ C_{16}-H), 7.35 (d, 1H, C_{17}-H, $J_{1,2}$ = 8 Hz), 7.44 (s , 1H, C_5-H), 7.97 (d, 1H, C_{18}-H, $J_{1,2}$ = 8.4 Hz): ^{13}C NMR (400 MHz, $CDCl_3$): δ 21.4 (C-23), 26.2 (C-24), 67.2 (C-11), 111.4 (C-3), 114.0 (C-14), 117.4 (C-16), 117.5 (C-8), 121.7 (C-15), 123.2 (C-18), 123.7 (C-22), 125.7 (C-10), 128.3 (C-7), 133.3 (C-5), 134.5 (C-6), 136.5 (C-17), 140.4 (C-21), 150.3 (C-9), 152.2 (C-13), 153.3 (C-19), 159.0 (C-4), 161.1 (C-2).

*7-Methyl-4-(2-methyl-quinolin-8-yloxymethyl)-chromen-2-one **(3f)***

Yield 95%; colorless solid; m.p. 256 - 260 ^{0}C; IR (KBr, cm^{-1}) 1721 cm^{-1} (lactone C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.42 (s, 3H, 7-CH_3), 2.79 (s, 3H, C_{19}-CH_3), 5.57 (s, 2H, OCH_2), 6.64 (s, 1H, C_3-H), 6.96 (d, 1H, C_6-H, $J_{1,2}$ = 7.6 Hz), 7.16 (d, 1H, C_5-H, $J_{1,2}$ = 6.4 Hz), 7.22 (d, 1H, C_{14}-H, $J_{1,2}$ = 6.8 Hz), 7.30 (d, 1H, C_{17}-H, $J_{1,2}$ = 8 Hz), 7.35 (m, 2H, $C_{15,}$ C_{16}-H), 7.42 (s , 1H, C_8-H), 8.0 (d, 1H, C_{18}-H, $J_{1,2}$ = 8.4 Hz).

*6-Methoxy-4-(2-methyl-quinolin-8-yloxymethyl)-chromen-2-one **(3g)***

Yield 85%; yellow solid; m.p. 197 - 199 ^{0}C; IR (KBr, cm^{-1}) 1704 cm^{-1} (lactone C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.80 (s, 3H, C_{19}-CH_3), 3.80 (s, 3H, OCH_3), 5.61 (s, 2H, OCH_2), 6.75 (s, 1H, C_3-H), 7.04 (d ,1H, C_7-H, $J_{1,2}$ = 6.8 Hz), 7.15 (d, 1H ,C_{14}-H, $J_{1,2}$ = 6.4 Hz), 7.24 (d, 1H, C_{16}-H, $J_{1,2}$ = 2.8 Hz), 7.31-7.36 (m, 3H, $C_{5,}$ C_8 & C_{15}-H), 7.44 (d, 1H, C_{17}-H, $J_{1,2}$ = 8.4 Hz), 8.10 (d ,1H, C_{18}-H, $J_{1,2}$ = 8.4 Hz).

*6-Ethyl-4-(2-methyl-quinolin-8-yloxymethyl)-chromen-2-one **(3h)***

Yield 95%; colorless solid; m.p. 264 - 266 ^{0}C; IR (KBr, cm^{-1}) 1734 cm^{-1}(lactone C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.26 (t, 3H, CH_3 of C_2H_5, $J_{1,2}$ = 7.6 Hz), 2.7 (q, 2H, CH_2 of C_2H_5), 2.81 (s, 3H, C_{19}-CH_3), 5.63 (s, 2H, OCH_2), 6.75 (s, 1H, C_3-H), 7.02 (d, 1H, C_{14}-H, $J_{1,2}$ = 7.2 Hz), 7.31-7.37 (m, 5H, C_7, C_8, C_{15}-H), 7.40-7.44 (m, 2H , C_{16} & C_{17}-H), 7.52 (s, 1H, C_5-H), 8.0 (d, 1H, C_{18}-H, $J_{1,2}$ = 8.4 Hz).

*6-Isopropyl-4-(-2-methyl-quinolin-8-yloxymethyl)-chromen-2-one **(3i)***

Yield 96%; colorless solid; m.p. 193 - 195 ^{0}C; IR (KBr, cm^{-1}) 1723 cm^{-1} (lactone C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.26 (d, 6H, 2-CH_3 of isopropyl, $J_{1,2}$ =6 Hz), 2.75 (s, 2H, C_{19}-CH_3), 2.95 (septd, 1H, CH of isopropyl, $J_{1,2}$ = 3 Hz), 5.62 (S, 2H, OCH_2), 6.70 (s, 1H, C_3-H), 7.04 (d, 1H, C_{16}-H, $J_{1,2}$ = 8.8 Hz), 7.30 (d, 1H , C_{14}-H, $J_{1,2}$ = 8.8 Hz), 7.39 - 7.43 (m, 2H, C_7 & C_{16}-H), 7.45-7.48 (m, 3H, C_5, C_{15} & C_{17}-H, $J_{1,2}$ = 4.4 Hz), 7.50(d, 1H, C_8-H, $J_{1,2}$ = 2 Hz), 8.15 (d, 1H, C_{18}-H, $J_{1,2}$ = 8.4 Hz).

*6-Tert-Butyl-4-(2-methyl-quinolin-8-yloxymethyl)-chromen-2-one **(3j)***

Yield 75%; brown solid; m.p. 179 - 182 ^{0}C; IR (KBr, cm^{-1}) 1718 cm^{-1}(lactone C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.36 (s, 9H, 6-tert-butyl), 2.80 (s, 3H, C_{19}-CH_3), 5.65 (s, 2H, OCH_2), 6.74 (s, 1H, C_3-H), 7.03 (d, 1H, C_7-H, $J_{1,2}$ = 7.2 Hz), 7.32-7.36 (m, 3H, C_8, C_{15} & C_{16}-H), 7.44 (d, 1H, C_{14}-H, $J_{1,2}$ = 7.2 Hz), 7.60 (d ,1H, C_{17}-H, $J_{1,2}$ = 6.4 Hz), 7.68 (s, 1H, C_5-H), 8.03 (d, 1H, C_{18}-H, $J_{1,2}$ = 8.4 Hz)

*6-Benzyl-4-(2-methyl-quinolin-8-yloxymethyl)-chromen-2-one **(3K)***

Yield 60%; colorless solid; m.p. 233 - 235 ^{0}C; IR (KBr, cm^{-1}) 1728 cm^{-1}(lactone C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.41 (s, 3H, C_{19}-CH_3), 2.78 (s, 2H, C_6-CH_2), 5.67 (s, 2H, OCH_2), 6.8 (s, 1H, C_3-H), 7.05-8.10 (m, 13H, Ar-H).

*1-(2-Methyl-quinolin-8-yloxymethyl)-benzo(f)chromen-3-one **(3l)***

Yield 85%; yellow solid; m.p. 197 - 199 ^{0}C; IR (KBr, cm^{-1}) 1723 cm^{-1}(lactone C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.81 (s, 3H, C_{19}-CH_3), 6.07 (s, 2H, OCH_2), 6.98-8.40 (m, C_3-H & 11H, Ar-H).

*6-Chloro-4-(2-methyl-quinolin-8-yloxymethyl)-chromen-2-one **(3m)***

Yield 70%; colorless solid; m.p. 216 - 218 ^{0}C; IR (KBr, cm^{-1}) 1712 cm^{-1}(lactone C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.80 (s, 3H, C_{19}-CH_3), 5.66 (s, 2H, OCH_2), 6.74 (s, 1H, C_3-H), 7.03 (d, 1H, C_8-H, $J_{1,2}$ = 7.2 Hz), 7.32-7.36 (m, 3H, C_7, C_{14} & C-$_{15}$H), 7.42 (d, 1H, C_{16}-H, $J_{1,2}$ = 7.2 Hz), 7.60 (d ,1H, C_{17}-H, $J_{1,2}$ = 6.4 Hz), 7.68 (s, 1H, C_5-H), 8.02 (d, 1H, C_{18}-H, $J_{1,2}$ = 8.4 Hz)

*6-Bromo-4-(2-methyl-quinolin-8-yloxymethyl)-chromen-2-one **(3n)***

Yield 73%; brown solid; m.p. 238 - 240 ^{0}C; IR (KBr, cm^{-1}) 1719 cm^{-1}(lactone C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.82 (s, 3H, C_{19}-CH_3), 5.55 (s, 2H, OCH_2), 6.81 (s, 1H, C_3-H), 7.07-8.06 (m, 8H, Ar-H).

*6- Fluoro-4-(2-methyl-quinolin-8-yloxymethyl)-chromen-2-one **(3o)***

Yield 65%; colorless solid; m.p. 226 - 228 ^{0}C; IR (KBr, cm^{-1}) 1704 cm^{-1}(lactone C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.80 (s, 3H, C_{19}-CH_3), 5.60 (s, 2H, OCH_2), 6.75 (s, 1H, C_3-H), 7.04 (d ,1H, C_7-H, $J_{1,2}$ = 6.8 Hz), 7.15 (d, 1H , C_{16}-H, $J_{1,2}$ = 6.4 Hz), 7.24 (d, 1H, C_{14}-H, $J_{1,2}$ = 2.8 Hz), 7.32-7.38 (m, 3H, C_5, C_8 & C_{15}-H), 7.44 (d, 1H, C_{17}-H, $J_{1,2}$ = 8.4 Hz), 8.10 (d ,1H, C_{18}-H, $J_{1,2}$ = 8.4 Hz).

Biological activity

The cytotoxic activity of newly synthesized compounds was examined *in vitro* on Dalton's Lymphoma Ascites (DAL) and Ehrlich Ascites Carcinoma (EAC) cells using Trypan blue dye exclusion assay.[19]

Ascitic fluid withdrawn from the peritoneum of DAL and EAC inoculated mouse was washed with ice cold phosphate buffer saline (PBS) (pH 7.4). Stock cell suspension was adjusted to $1{\times}10^6$ cell / 0.2 mL by PBS using hemocytometer. The cells were incubated with desired test drug concentration in a final volume of 1mL for 3h at 37 ^{0}C in CO_2 incubator with continuous flow of 5% CO_2, *5-Fluorouracil* was used as positive control. After incubation, 0.2 mL of cell line was taken and made up to final concentration with PBS (0.3 mL), trypan blue (0.5 mL), mixed well and kept aside for 5 min. The total number of dead and living cells was counted using a hemocytometer and the percentage viability or cytotoxicity was calculated. All the procedures were done in triplicate manner.

RESULTS AND DISCUSSION

In the IR spectrum of 4-bromomethyl-6-ethylcoumarin **(1d)** (R = 6-ethyl), the lactone carbonyl stretching frequency was observed at 1701 cm^{-1}. The ^{1}H NMR spectrum of compound **(1d)** exhibited a singlet at δ 4.50, 6.51 and 7.50 due to CH_2-Br, C_3-H and C_5-H protons respectively. A triplet and a quartet were observed at δ 1.26 ($J_{1,2}$ = 8 Hz) and 2.71 ($J_{1,2}$ = 7.6 Hz), due to methylene and methyl protons of ethyl group. The C_7-H and C_8-H protons were found to resonate as a doublet at δ 7.39 ($J_{1,2}$ = 10.4 Hz) and 7.26 ($J_{1,2}$ = 5.2 Hz) respectively.

In the IR spectrum of 6-methyl-4-(2-methyl-quinolin-8-yloxymethyl)-chromen-2-one **(3e)** (R = 6-CH_3, R_1= CH_3), the lactone carbonyl stretching frequency was appeared at 1716 cm^{-1}. The ^{1}H NMR spectrum of the compound **(3e)** displayed a singlet at δ 2.38, 5.55, 6.68 and 7.44 due to C_{19}-CH_3, OCH_2, C_3-H and C_5-H protons respectively. The C_7, C_8, C_{14}, C_{17} and C_{18}-H protons resonated as a doublet at δ 6.96 ($J_{1,2}$ = 7.6 Hz), 7.20 ($J_{1,2}$ = 6.4 Hz), 7.23($J_{1,2}$ = 6.8 Hz), 7.37($J_{1,2}$ = 8 Hz) and 7.99($J_{1,2}$ = 8.4 Hz) respectively. The multiplet appeared in the range of 7.32-7.35 was due to C_{15}-H and C_{16}-H respectively.

The ^{13}C NMR spectral data of compound (**3e)** is given in the method and materials section, which is confirmed by its HSQC spectrum.

***In vitro* anticancer screening**

The newly synthesized compounds **(3a-o)** were evaluated for their *in vitro* cytotoxic activity against Dalton's Lymphoma Ascites (DAL) and Ehrlich Ascites Carcinoma (EAC) Cells. *5-Fluorouracil* which is one of the most effective anticancer agents 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 Lymphoma Ascites (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 result is represented graphically in Figure 2.

Table 1: Result of *in vitro* cytotoxic activity of the synthesized compounds against DAL & EAC cells.

Compound	R	R_1	DAL IC_{50}	EAC IC_{50}
3a	6-C_2H_5	H	70.58	59.20
3b	6-i-Pr	H	97.19	79.26
3c	6-tert-butyl	H	78.54	63.47
3d	6-Benzyl	H	45.85	93.99
3e	6-CH_3	CH_3	74.21	59.26
3f	7-CH_3	CH_3	150.48	48.84
3g	6-OCH_3	CH_3	140.87	46.26
3h	6-C_2H_5	CH_3	143.25	78.24
3i	6-i-Pr	CH_3	54.28	39.26
3j	6-tert-butyl	CH_3	74.68	46.20
3k	6-Benzyl	CH_3	154.25	62.99
3l	5,6-Benzo	CH_3	107.88	96.20
3m	6-Cl	CH_3	67.81	78.65
3n	6-Br	CH_3	54.25	109.34
3o	6-F	CH_3	65.24	61.26
5-Fluorouracil			41.61	41.61

The investigation of *in vitro* cell cytotoxicity against DAL cell revealed that the most of the tested compounds exhibited good activity. The Compound (**3d**) (R = 6-Benzyl, R_1 = H) was the most potent compound in this screening against DAL cell with IC_{50} value of 45.86 µg/mL. The compounds **(3i)** (R = 6-i-Pr, R_1 = CH_3), **(3m)** (R = 6-Cl, R_1 = CH_3), **(3n)** (R = 6-Br, R_1 = CH_3) and **(3o)** (R = 6-F, R_1 = CH_3) were found to be highly active against DAL cell with IC_{50} between 54.25 and 67.81 µg/mL. The compounds **(3a)** (R = 6-Ethyl, R_1 = H), **(3b)** (R = 6-i-Pr, R_1 = H), **(3c)** (R = 6-tert-butyl, R_1 = H), **(3e)** (R = 6-CH_3, R_1 = CH_3) and **(3j)** (R = 6-tert-butyl, R_1 = CH_3) showed moderate activity against DAL cell with IC_{50} between 74.21 and 97.19 µg/mL. The compounds **(3f)** (R = 7-CH_3, R_1 = CH_3), **(3g)** (R = 6-OCH_3, R_1 = CH_3), **(3h)** (R = 6-Ethyl, R_1 = CH_3), **(3k)** (R = 6-Benzyl, R_1 = CH_3) and **(3l)** (R = 5,6-Benzo, R_1 = CH_3) showed poor activity against DAL cell with IC_{50} between 107.88 and 150.48 µg/mL.

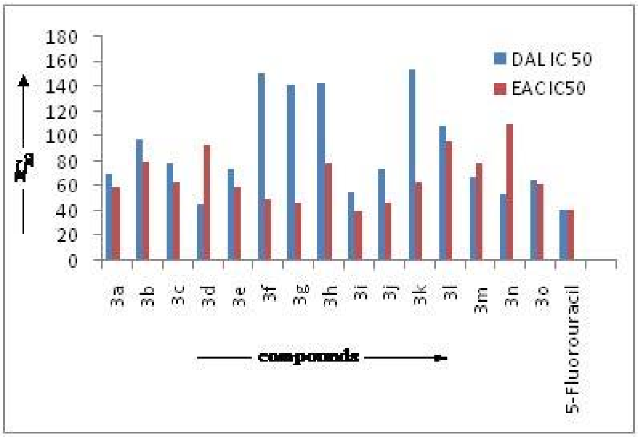


Figure 2: IC_{50} µg/mL of the synthesized compounds and 5-Flourouracil against DAL & EAC cells.

The investigation of *in vitro* cell cytotoxicity against EAC cell revealed that the most of the tested compounds exhibited good activity. The Compound **(3i)** (R = 6-i-Pr, R_1 = CH_3) was found to be highly active against EAC cell with IC_{50} value of 39.26 µg/mL. The compounds **(3a)** (R = 6-Ethyl, R_1 = H), **(3c)** (R = 6-tert-butyl, R_1=H), **(3e)** (R = 6-CH_3, R_1 = CH_3), **(3f)** (R = 7-CH_3, R_1 = CH_3), **(3g)** (R = 6-OCH_3, R_1 = CH_3), **(3j)** (R = 6-tert-butyl, R_1 = CH_3), **(3k)** (R = 6-Benzyl, R_1 = CH_3) and **(3o)** (R = 6-F, R_1 = CH_3) were found to be highly active against EAC cell with IC_{50} between 46.20 and 63.47 µg/mL. The compounds **(3b)** (R = 6-i-Pr, R_1 = H), (**3d**) (R = 6-Benzyl, R_1=H), **(3h)** (R = 6-Ethyl, R_1=CH_3), **(3l)** (R = 5,6-Benzo, R_1 = CH_3) and **(3m)** (R = 6-Cl, R_1 = CH_3) showed moderate activity against EAC cell with IC_{50} between 78.24 and 96.20 µg/mL. The compounds **(3n)** (R= 6-Br, R_1 = CH_3) showed poor activity against EAC cell with IC_{50} 109.34 µg/mL.

CONCLUSION

Introduction of benzyl group at 6-position of coumarin ring is found to enhance the cytotoxicity against DAL cell and also, introduction of isopropyl group at 6-position of coumarin ring and methyl group at 2-position of quinoline ring is found to enhance the cytotoxicity against EAC cell.

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