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Design and synthesis of certain novel bicoumarin derivatives as anticancer agents

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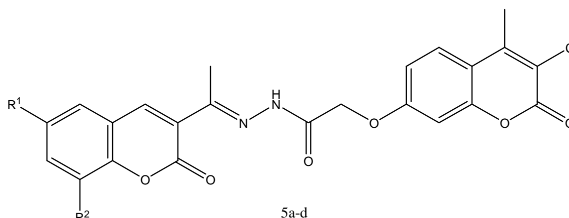
ABSTRACT

New Bicoumarin derivatives ***5a-c****were synthesized through condensation of 3-acetylcoumarin derivatives with coumarin acid hydrazide. All the prepared compounds were evaluated for their in vitro anticancer activity against human hepatic (Hep-G2) and human breast (MCF-7)cancer cell lines. The prepared compounds showed variable anticancer activity against Hep-G2 cell line with compound* ***5c*** *being the most potent with IC_{50} 1.21 mg/mL.Also, compound* ***5a*** *showed moderate activity with IC_{50} 4.62 mg/mL against the same cell line. However, all the prepared compounds showed no activity against MCF-7 cell line with IC_{50}>10mg/mL.*

Keywords: Bicoumarin, Hep-G2, MCF-7.

INTRODUCTION

Cancer is a serious clinical problem that has significant socioeconomical effects on the human healthcare and is the second leading cause of death in most countries after cardiovascular diseases[1].All human cancers have six acquired capabilities that enable malignant growth (Fig.1) [2].Tumor growth and expansion require not only an ability to proliferate, but also to resist apoptosis and activate angiogenesis to produce a tumor neovasculature[3].



Thus, The development of new, effective, selective and less toxic compounds has become anew approach for discovery of cytotoxic agents that promote apoptosis and inhibit angiogenesis of cancer cells[1].

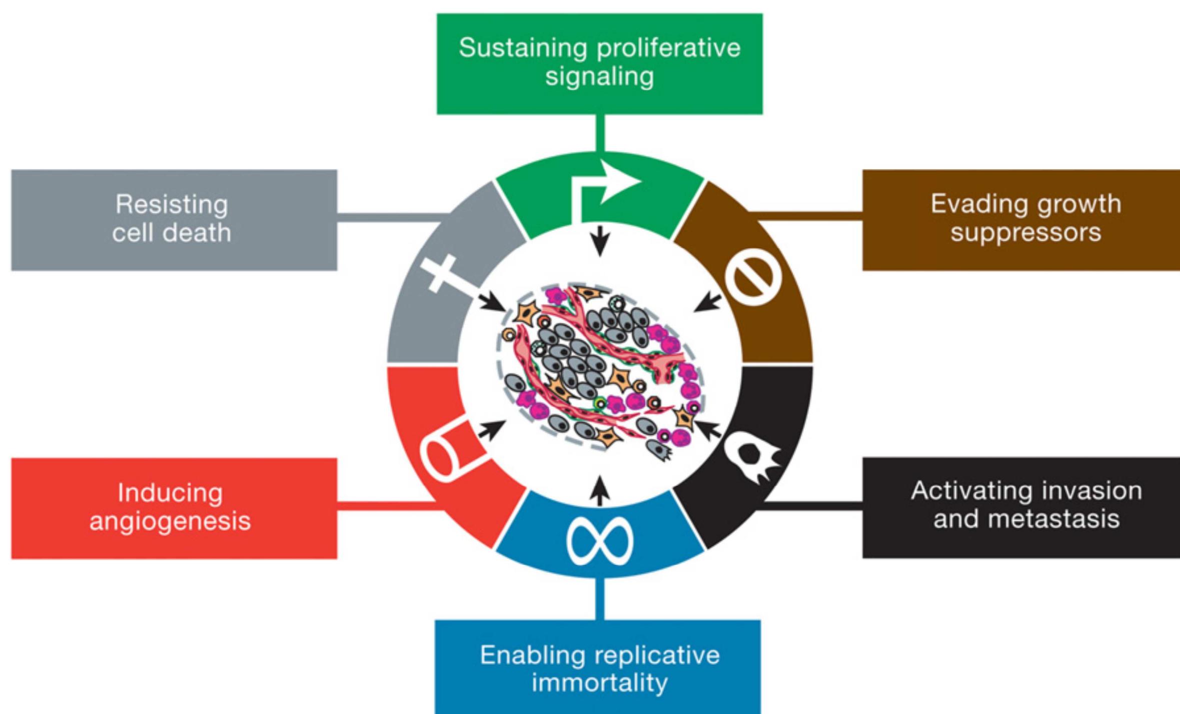


Fig.1: The six hallmarks of cancer

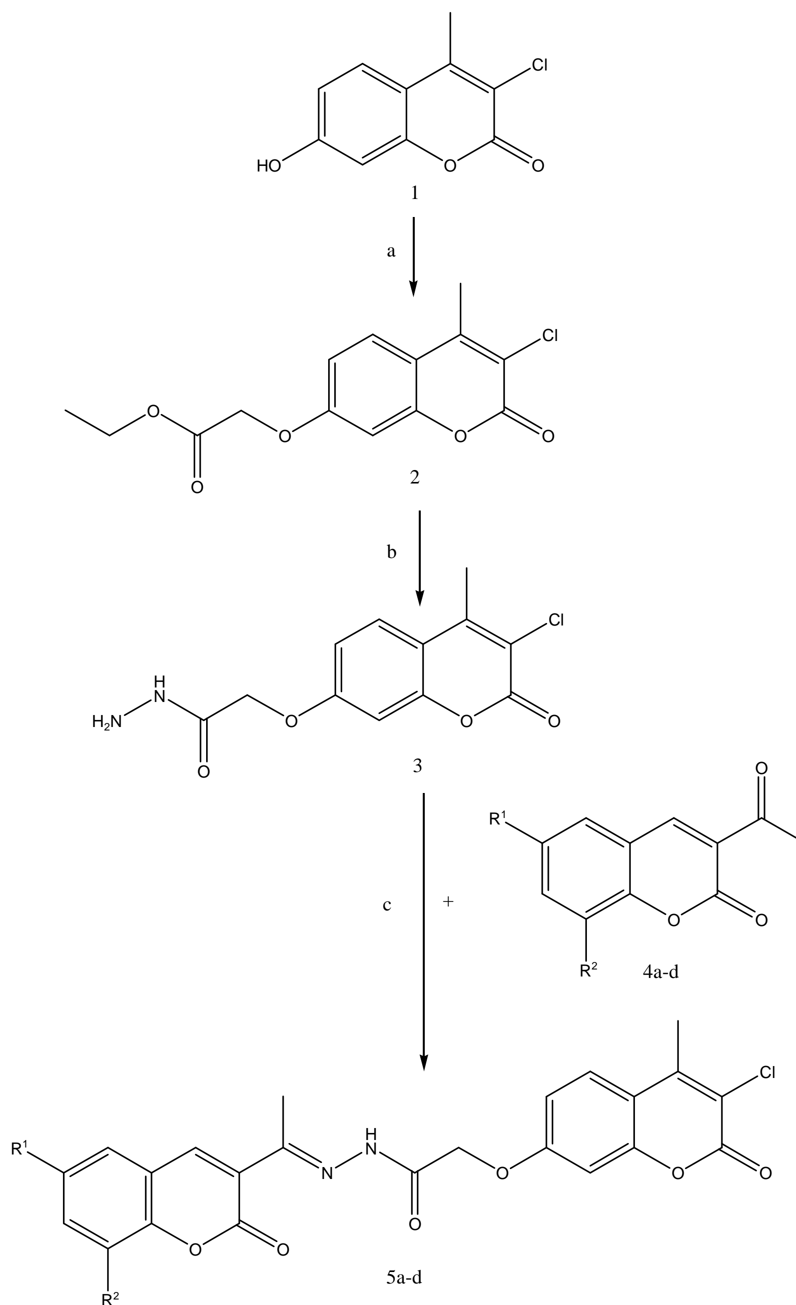
Natural and synthetic coumarins attract great attention due to their diverse biological activities, including anticancer[4, 5], anti-HIV [6], anti-inflammatory [7, 8], analgesic [8], antituberculosis [8], antibacterial [8],antioxidant [9], anticoagulant[10],and antimicrobial [11]activities. Among these activities, their anticancer effects were the most extensively examined. Coumarins could exert their anticancer activity by different mechanisms [12-16]; Studies have revealed that the mechanism behind the anticancer effect of coumarin analogs may be due to inhibition of angiogenesis and induction of apoptosis [17-22].Hence, it is very essential to synthesize and develop novelcoumarin analogs with multiple targets of cytotoxic actions. In the present study efforts have been made to synthesize novel derivatives of bicoumarin analogs(Scheme 1) and evaluate their cytotoxic effect against human breast (MPC-7) and human hepatic (Hep-G2) cancer cell lines. Based on the previous information, and in continuation of our previous work [23-25] for development of new anticancer agents,we now describethe synthesis and *in vitro* cytotoxic investigation of bicoumarin analogs (Scheme 1) against human breast (MPC-7) and human hepatic (Hep-G2) cancer cell lines.

MATERIALS AND METHODS

Chemistry

Melting points were determined on a Griffin apparatus and were uncorrected. Infrared (IR) spectra were recorded on a Shimadzu 435 Spectrometer, using KBr discs and values were represented in cm^{-1}. ^{1}H-NMR and ^{13}C-NMR spectra were carried out on a Bruker 400 MHz spectrometer in Faculty of pharmacy BeniSuef University, Egypt. Samples were dissolved in $CDCl_3$-d or DMSO-d_6 with TMS as the internal standard, where J (coupling constant) values are estimated in hertz (Hz) and chemical shifts were recorded in ppm on δ scales. Mass spectra were run on a Hewlett Packard 5988 spectrometer at the regional center for mycology and biotechnology, Al-Azhar University, Egypt. Element analysis was carried out for C, H, N at the regional center for mycology and biotechnology, Al-Azhar University, Egypt. Progress of the reactions was monitored using thin layer chromatography (TLC) sheets that precoated with UV fluorescent silica gel MERCK 60 F 254 that was visualized by UV lamp. Solvent system was chloroform: methanol (in different ratio).All chemicals purchased from the Aldrich Chemical Company (Milwaukee, WI), were used without further purification.

Compounds **(1)**was purchased, while**(2, 3, 4a-d)**were prepared according to the reported procedures [26, 28-30].



Scheme 1: reagents and coditions: (a) Ethyl chloroacetate, DMF, K_2CO_3, reflux, 2h. (b) hydrazine hydrate, reflux, 6h. (c) glacial acetic acid, reflux, 4 h.

No.	R^1	R^2
4a, 5a	H	H
4b, 5b	H	OCH_3
4c, 5c	H	OC_2H_5
4d, 5c	Br	OCH_3

General procedure for synthesis of (*ZE*)-2-((3-chloro-4-methyl-2-oxo-2H-chromen-7-yl)oxy)-*N′*-(1-(2-oxo-2*H*-chromen-3-yl)ethylidene)acetohydrazide derivatives 5a-d:mixture of 2-((3-chloro-4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetohydrazide(**3**) (0.01 mol, 2.82 gm) and appropriate acetyl coumarin derivative **4a-d**(0.01 mol) in 20

mL glacial acetic acid was heated under reflux for 4h. The obtained solid product was filtered while hot, washed several times with hot methanol and crystalized from isopropanol to yield compounds**5a-d.**

(*ZE*)-2-((3-chloro-4-methyl-2-oxo-2H-chromen-7-yl)oxy)-*N′*-(1-(2-oxo-2*H*-chromen-3-yl)ethylidene) acetohydrazide(5a)83% yield; white solid; mp 238-240°C; IR (KBr disc)3250 (NH), 3065 (CH aromatic), 2924 (CH aliphatic), 1742 (C=O acetyl coumarin), 1703 (C=O coumarin hydrazide), 1612 (C=O amide) cm^{-1}; ^{1}HNMR (*DMSO-d_6*): δ 2.22 (s,3*H*, CH_3hydrazone), 2.55 (s, 3H, CH_3on C4 coumarine), 4.95 (s,0.6*H*, CH_2), 5.34 (s, 1.4*H*, CH_2), 7.04 (s, 1*H*, *H*8), 7.07 (d, *J* = 6.8, 1*H*, *H*6), 7.40 (t, *J* = 7.6, 1*H*, *H'*6), 7.45 (d, *J* = 7.6, 1*H*, *H'*8), 7.66 (t, *J* = 7.6, 1*H*, *H'*7), 7.80 (m, 2*H*, *H*5&*H'*5), 8.20 (s, 0.3*H*, *H'*4), 8.36 (s, 0.7*H*, *H'*4), 10.77 (s, 0.3*H*, NH, *D_2O* exchangeable), 11.08 (s, 0.7*H*, NH, *D_2O* exchangeable);EIMS (m/z):454.04 (M+2, 3.33), 453.07 (M+1, 1.95), 452.03 ($M^{+\cdot}$, 7.33), 107 (100%); Anal.calcd. for $C_{23}H_{17}ClN_2O_6$: C, 61.00; H, 3.78; N, 6.19; found: C, 61.18; H, 3.84; N, 6.34.

(*ZE*)-2-((3-chloro-4-methyl-2-oxo-2H-chromen-7-yl)oxy)-*N′*-(1-(8-methoxy-2-oxo-2*H*-chromen-3-yl) ethylidene) acetohydrazide (5b) 86% yield; white powder; mp242-244°C; IR (KBr disc) 3253 (NH), 3070 (CH aromatic), 2938 (CH aliphatic), 1742 (C=O acetyl coumarin), 1704 (C=O coumarin hydrazide), 1610 (C=O amide), 1272 (O-CH_3 ether linkage) cm^{-1}; ^{1}HNMR (DMSO-*d_6*); *δ*2.20 (s, 2.1*H*, CH_3hydrazone), 2.26 (s, 0.9*H*, CH_3hydrazone), 2.53 (s, 3*H*, CH_3 hydrazide), 3.92 (s, 3*H*, OC$\underline{H_3}$), 4.94 (s, 0.6*H*, CH_2), 5.33 (s, 1.4*H*, CH_2), 7.02 (s, 1*H*, *H*8), 7.05 (d, *J* = 8.8, 1*H*, *H*6), 7.33 (s, 3*H*, *H'*5-7), 7.77 (d, *J* = 8.8, 1*H*, *H*5), 8.11 (s, 0.3*H*, *H'*4), 8.32 (s, 0.7*H*, *H'*4), 10.79 (s, 0.3*H*, NH, *D_2O* exchangeable), 11.12 (s, 0.7*H*, NH, *D_2O*exchangeable); EIMS (m/z):484.54 (M+2, 0.75), 483.76 (M+1, 0.93), 482.17 ($M^{+\cdot}$, 1.58), 150(100%); Anal.calcd. for$C_{24}H_{19}ClN_2O_7$: C, 59.70; H, 3.97; N, 5.80;. found: C, 59.87; H, 4.03 ; N, 5.89.

(*ZE*)-2-((3-chloro-4-methyl-2-oxo-2H-chromen-7-yl)oxy)-*N′*-(1-(8-ethoxy-2-oxo-2*H*-chromen-3-yl) ethylidene) acetohydrazide (5c):76% yield; white solid;mp251-253°C; IR (KBr disc)3253 (NH), 3070 (CH aromatic), 2938 (CH aliphatic), 1704 (2 C=O coumarine), 1610 (C=O amide), 1272 (O-C_2H_5 ether linkage) cm^{-1}; ^{1}HNMR (DMSO-*d_6*): δ 1.42 (t, *J*=6.8, 3*H*, $CH_2C\underline{H_3}$), 2.20 (s, 2.1*H*, CH_3hydrazone), 2.26 (s, 0.9*H*, CH_3hydrazone), 2.54 (s, 3*H*, CH_3on C4coumarin), 4.19 (q, *J* = 6.8, 2*H*, $OC\underline{H_2}CH_3$), 4.94 (s, 0.6*H*, CH_2), 5.33 (s, 1.4*H*, CH_2); 7.04 (s, 1*H*, *H*8), 7.05 (d, *J* = 8.4, 1*H*, *H*6), 7.32 (m, 3*H*, *H'*5-7), 7.78 (d, *J* = 8, 1*H*, *H*5), 8.16 (S, 0.3*H*, *H'*4), 8.32 (S, 0.7*H*, *H'*4), 10.77 (S, 0.3*H*, NH, *D_2O* exchangeable), 11.07 (S, 0.7*H*, NH, *D_2O* exchangeable); EIMS (m/z): 498.09 (M+2, 3.27), 496.09 ($M^{+\cdot}$, 6.36),87.99(100%); Anal.calcd. for $C_{25}H_{21}ClN_2O_7$: C, 60.43; H, 4.26; N, 5.64; found: C, 60.60; H, 4.31; N, 5.78.

(ZE)-2-((3-chloro-4-methyl-2-oxo-2H-chromen-7-yl)oxy)-N'-(1-(6-bromo-8-methoxy-2-oxo-2H-chromen-3-yl) ethylidene) acetohydrazide (5d):79% yield; pink solid; mp237-239°C; IR (KBr disc) 3258 (NH), 3071 (CH aromatic), 2927 (CH aliphatic), 1704 (2 C=O coumarine), 1611 (C=O amide), 1274 (O-CH_3 ether linkage) cm^{-1}; ^{1}HNMR ($CDCl_3$): δ 2.21 (s, 2.1*H*, CH_3hydrazone), 2.25 (s, 0.9*H*, CH_3hydrazone), 2.55 (s, 3*H*, CH_3 hydrazide), 3.95 (s, 3*H*, OC$\underline{H_3}$), 4.94 (s, 0.6*H*, CH_2), 5.31 (s, 1.4*H*, CH_2), 7.03 (s, 1*H*, *H*8), 7.05 (d, *J* = 8.8, 1*H*, *H*6), 7.50 (s, 1*H*, *H'*5), 7.58 (s, 1*H*, *H'*7); 7.79 (d, *J* = 8.8, 1*H*, *H*5), 8.11 (s, 0.3*H*, *H'*4), 8.28 (s, 0.7*H*, *H'*4), 10.79 (s, 0.3*H*, NH, *D_2O* exchangeable); 11.12 (s, 0.7*H*, NH, *D_2O* exchangeable).; EIMS (m/z): 564.63 (M+2, 2.17), 563.07 (M+1, 4.03), 562.00 ($M^{+\cdot}$, 10.21), 178.03(100%); Anal.calcd. for $C_{24}H_{18}BrClN_2O_6$: C, 51.31; H, 3.23; N, 4.99; found: C, 51.49; H, 3.21; N, 5.12.

Pharmacological studies

Cell Culture

The two human cancer cell lines used in this study breast carcinoma (MCF-7) and hepatic carcinoma (HepG-2) were purchased from American Type Culture Collection. Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM: Gibco, USA) supplemented with 10% fetal bovine serum (Gibco), penicillin/streptomycin (Gibco).4',6-Diamidino-2-phenylindole (DAPI) nuclear staining indicated that these cells were devoid of mycoplasma contaminations. All incubations were done at 37°C in a humidified incubator containing 5% CO_2. Cells in log-phase growth were harvested by trypsinization for use in various assays.

Cell Viability Analysis

Cell viability was assessed using MTT assay (Sigma-Aldrich, St. Louis, MO, USA) in 6 replicates as described previously[31].In brief, a total of $1{\times}10^4$ cells per well were seeded into 96-well tissue culture plates in DMEM containing 10% FBS to a final volume of 0.2 ml. The cells were subjected to different treatments 24 h post seeding.

Following the incubation for 48 h with doxorubicin and cisplatin (positive control), test drugs or vehicle (DMSO), the media were removed, replaced by 200 µL DMEM containing 0.5 mg/mL of MTT and cells were incubated for 2 h. Next, the supernatants were removed and the precipitated formazan was dissolved by adding 200 µl of DMSO. Absorbance at 570 nm was determined using a microplate reader (Model 450 Mioroplate Reader; Bio-Rad).Results were calculated by subtracting blank readings.

RESULTS AND DISCUSSION

Chemistry

Formation of the target bicoumarin compounds **5a-d** is explained in terms of condensation of 3-acetyl coumarin derivatives**4a-d** with coumarin acid hydrazide derivative **3** as outlined in Scheme 1. The synthetic procedure was carried out by heating a mixture of (4-Methyl-2-oxo-2H-chromen-7-yloxy)-acetic acid hydrazide**(3)** and3-acetyl coumarin derivatives **4a-d** in glacial acetic acid under reflux for 4 h. The structure of **5a-d**was confirmed by spectral analysis (^{1}HNMR, IR and mass spectroscopy) and element analyses. IR spectra showed strong band at3258-3250 cm^{-1} indicating the presence of NH group. Also, absorption bands at 1742-1703and1612-1610cm^{-1} due to the presence of two CO group ofα-lactone and CO group of amide, respectively.

The ^{1}H NMR spectra of the target compounds revealed the appearance of Z & E isomer by the appearance of two adjacent single peaks at δ 4.94-4.95 and 5.31-5.33 ppm equal to 0.6 and 1.4 protons, respectively attributed to ($OC\underline{H_2}$) and two peaks at δ 8.11-8.20 and 8.28-8.36ppmequal to 0.3 and 0.7 proton, respectively indicating *H*4 pyrone ring. Also, two adjacent peaks which was D_2O exchangeable at δ 10.77-10.79 and 11.07-11.12 ppm equal to 0.3 and 0.7 proton, respectively indicating NH proton.

Also, mass spectra and elemental analysis confirmed the formation of compounds (**5a-d).**

Compound 3 was prepared from 3-Chloro-7-hydroxy-4-methyl-chromen-2-one(**1**)in two steps according to reported method [26]. First step depends on alkylation of phenolic OH group with ethyl chloroacetate by heating in DMF containing anhydrous potassium carbonate under reflux for 2h. The ester formed **(2)** is treated with hydrazine hydrate in and heated in ethanol under reflux for 4h to afford the acid hydrazide(**3**).

Biological evaluation

All the synthesized derivatives, cisplatin and doxorubicin [as reference drugs control] were *in vitro* evaluated for their cytotoxic activity against human breast (MCF-7) and human hepatic (Hep-G2) cancer cell lines, using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay[27].

The half maximal inhibitory concentration (IC_{50}) was calculated for compounds **5a-d**and reference drugs (doxorubicin and cisplatin) to investigate the effect of structural changes on the activity as shown in table 1.

Table 1.IC_{50} of the synthesized compounds against MCF-7 and Hep-G2 cancer cell lines

Compounds	**IC_{50}(mg/mL)**	
	MCF-7	**Hep-G2**
5a	>10	4.62
5b	>10	9.68
5c	>10	1.21
5d	>10	>10
Cisplatin	4.5084	3.5738
Doxorubicin	2.0143	2.5738

The obtained data revealed that, all the prepared compounds have no activity against human breast MCF-7 cancer cell line. Among the tested compounds, bicoumarin derivative **5c** showed the most potent cytotoxic effect against Hep-G2 cell lines with IC_{50} value of 1.21mg/mL. In addition, compound **5a** showed moderate activity againstHep-G2 cell line with IC_{50}4.62mg/mL, compared with cisplatin and doxorubicin as reference drugs that have IC_{50}3.5738 and 2.5738 mg/mL, respectively against the same cell line **(Figure 2)**.

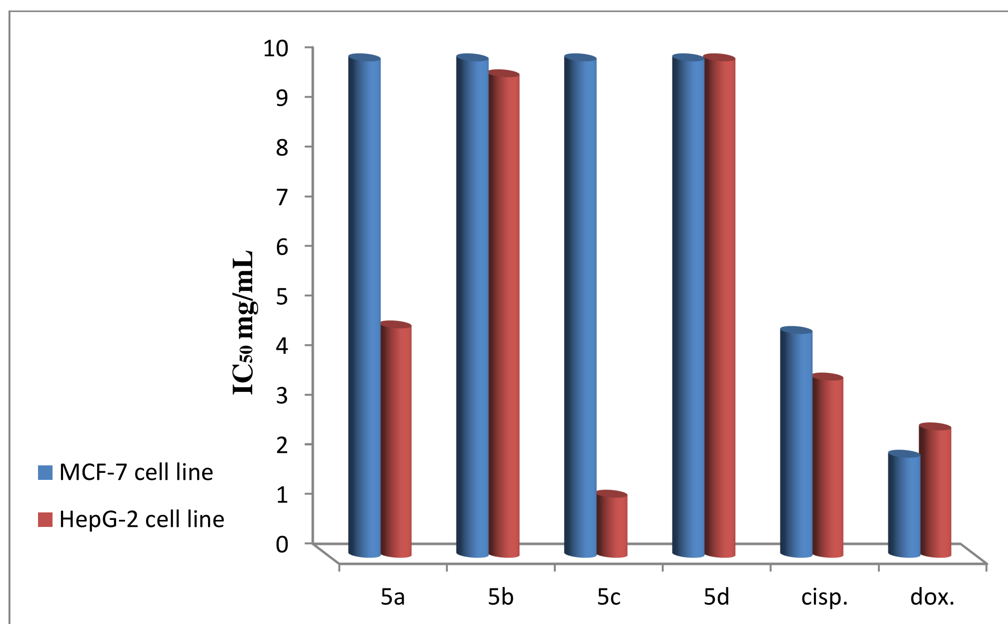


Fig.2A graph representing IC_{50} (mg/mL) against (MCF-7) and (Hep-G2) cell lines for compounds (5a -d), Cisp. and Dox

CONCLUSION

Four new bicoumarin derivatives had been synthesized and evaluated for their *in vitro* antitumor activity against human breast MCF-7 and human hepaticHep-G2 cancer cell lines. It was concluded that compound **5c** is the most potent anticancer agent with IC_{50} value of 1.21mg/mL against human hepatic cancer HepG-2 cell lines. In addition, compound **5a** moderate anticancer activity with IC_{50} 4.62 mg/mL respectively against the same cell line. However, none of the prepared compounds showed activity against MCF-7 human breast cancer cell line.

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