Original Research Article

Cytotoxicity of N-(P-chlorophenyl)-7-hydroxycoumarin-3-yl carboxamide and Ethyl 7-hydroxycoumarin-3-yl ester

Abstract:

Background: Coumarins (2H-1-benzopyran-2-one), an important class of heterocyclic compounds, and its derivatives can be found in many natural or synthetic drug molecules and possess versatile bioactivities making them important molecules for medical practitioners and medicinal chemists.

Aims and Objective: Our study aims to evaluate cytotoxicity of new Coumarin derivatives: N-(P-chlorophenyl)-7-hydroxycoumarin-3-yl carboxamide and Ethyl 7-hydroxycoumarin-3-yl ester against four human cell lines such as human breast cancer (MCF-7), human liver cancer (HEPG-2), human colon cancer (HCT) and human prostate cancer cell (PC-3).

Methodology: The ethyl-7-hydroxycoumarin-3-ylester (comp-1) was prepared via cyclocondensation of 2, 4-dihydroxybenzaldhyde with diethylmalonate in the presence of piperidine under fusion followed by Amonolyses with 4-chloro-aniline in the presence of acid medium under fusion produced the N-(4-chlorophenyl)-7-hydroxycoumarin-3-yl carboxamide (comp-2).

Result: The synthesized compounds have potent cytotoxicity against different tumor cell lines (MCF-7, HEPG-2, HCT, and PC-3).

Discussion: The compound N-(P-chlorophenyl)-7-hydroxycoumarin-3-yl carboxamide is better than Ethyl 7-hydroxycoumarin-3-yl ester compound because of the nature of the halogen atom (a chlorine or a bromine atom) in the 'meta' position of the phenyl ring relative to the ester oxygen atom of 2-oxo-2H-1-benzopyran- 3-carboxylate led to a better anti-tumor effect than that observed in the absence of any substituent.

Keywords: Coumarins, cytotoxicity, tumor cell lines.

1- Introduction:

Cancer is a disease characterized by abnormal cell proliferation and can invade other parts of the body through circulation. It is also known as a malignant tumor or malignant neoplasm. Many therapeutic anticancer have been developed which has relied on surgery, chemotherapy, radiotherapy, hormone therapy and immunotherapy **(Khorshid., 2011)**. Therefore the search for potent, safe and selective anticancer compounds is a crucial aspect of modern cancer research **(Vani *et al*., 2010)**. The side

effects of Chemotherapy are usually caused by its effects on healthy cells. Consequently, the principal obstacles to the clinical efficacy of chemotherapy remain their possible toxicity to normal tissues of the body, beside the development of cellular drug resistance especially to conventional anticancer agents **(Sherif ., 2010)**.

Natural or synthetic coumarins due to their wide range of biological activities have become an interesting subject of investigation for many researchers. Coumarin scaffold has proven to have an important role in anticancer drug development due to a fact that many of its derivatives have shown an anticancer activity on various cell lines. Action of coumarins on tumor cells is executed by different mechanisms and some of them show very good selectivity towards the cancer cells (**Klenkar *et al.*, 2015).**

Coumarins belong to benzopyrone chemical class, more precisely benzo-α-pyrones, where benzene ring is fused to pyrone ring (**Lacy *et al.*, 2004)**. In nature, Coumarins are found in higher plants like *Rutaceae* and *Umbelliferae* and some essential oils like Cinnamon barf oil, Cassia leaf oil and Lavender oil are also rich in coumarins. Except from higher plants, coumarins were found in microorganisms as well, like novobiocin and coumermycin from *Streptomyces* and aflatoxins from *Aspergillus* species **(Jain *et al.*, 2012).**

Coumarins are proven to possess a wide range of biological activities, anti-influenza **(Yeh *et al.*, 2010),** anti-inflammatory **(Lee *et al.*, 2011)**,antioxidant **(Kostova *et al.*,2011)**, antitumor **(Huang *et al.*, 2011),**antituberculosis**(Manvar *et al.*, 2011)**, antimicrobial **(Nitiema *et al.*, 2012)**, antinociceptive, anti- Alzheimer **(Anand *et al.*, 2012)**, antiasthmatic **(Sanchez-Recillas *et al.*, 2014)**, antiviral **(Xu *et al.*, 2014),** anti-HIV **(Kudo *et al.*, 2015)** , antidepressant **(Sashidhara *et al.*, 2015)**, , antihyperlipidemic **(Asif, 2015)**.

Antitumor activity of natural and synthetic coumarin derivatives have been extensively explored by many researchers **(Wang *et al.*, 2015)** and it has been proven that coumarins, depending on their structure, can act on various tumor cells by different mechanisms; they inhibit the telomerase enzyme, protein kinase activity and down regulating oncogene expression or induce the caspase-9-mediated apoptosis, suppress cancer cell proliferation by arresting cell cycle in G0/G1 phase, G2/M phase and affecting the p-glycoprotein of the cancer cell **(Amin *et al.*, 2013;Nasr *et al.*, 2014)**.

Coumarin derivatives can possess not only cytostatic, but cytotoxic properties as well **(Benci *et al.*, 2012)**. **(Marshall *et al.*, 1991)** showed that coumarin and 7-hydroxyycoumarin can inhibit growth in human cancer cell lines such as A549 (lung), ACHN (renal), H727 (lung), MCF7 (breast) and HL-60 (leukaemia) and in some clinical trials they exhibited anti-proliferative activity in prostate cancer **(Mohler *et al.*, 1992)**, malignant melanoma **(Thornes *et al.*, 1994).**

Coumarins also exhibited the cytotoxic effect against Hep2 cells (human epithelial type 2) in dose dependent manner and showed some typical characteristics of apoptosis with loss of membrane microvilli, cytoplasmic hyper-vacualization and nuclear fragmentation **(Mirunalini *et al*., 2014)**.

Our study aims to evaluate the cytotoxicity properties of recently developed synthetic coumarin derivatives: N-(P-chlorophenyl)-7-hydroxycoumarin-3-yl carboxamide and Ethyl 7-hydroxycoumarin-3-yl ester against the different tumor cell line Such as MCF-7, HEPG-2, HCT, and PC-3 cell lines.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

2, 4-dihydroxybenzaldehyde, Diethylmalonate, piperidine, ethanol, Hydrochloric acid (2%), p-chloroaniline, acetic acid were obtained from El-Gomhoria Chemical Co. Port-said. All chemicals were used as received without extra purification.

2.1.2. Cell culture

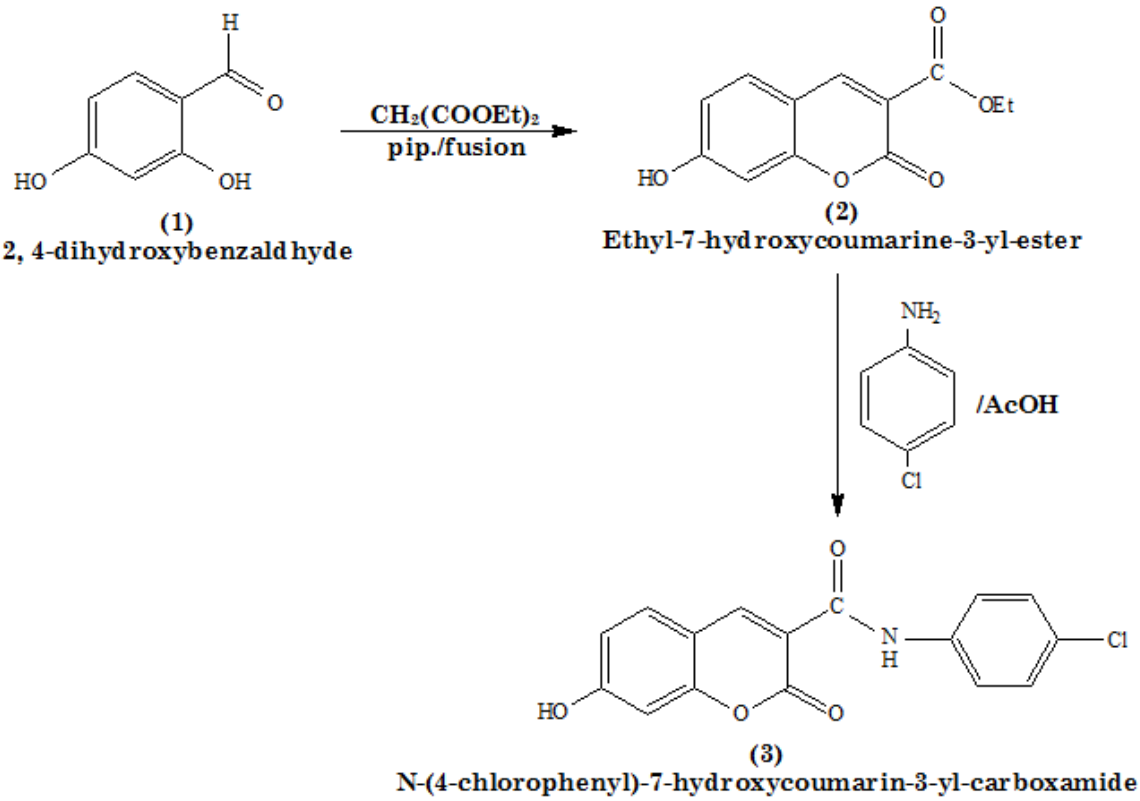
Cancer cells from different cancer cell lines, human breast adenocarcinoma (MCF-7), human hepatocellular carcinoma (HEPG-2), human colon adenocarcinoma (HCT-116) and human prostate cancer cells (PC-3) were purchased from American Type Culture Collection (ATCC, Manassas, USA) and grown on Roswell Park Memorial Institute Medium (PRMI 1640) supplemented with 100mg/ ml of streptomycin, 100 unites / ml of penicillin and 10% of heat-inactivated fetal bovine serum in humidified ,5% (v/v) CO_2 atmosphere at 37 ^{o}C.

2.2. Methods

2.2.1. Chemistry

The ethyl-7-hydroxycoumarin-3-ylester (comp-1) was prepared via cyclocondensation of 2, 4-dihydroxybenzaldhyde with diethylmalonate in the presence of piperidine under fusion according to a literature method (**El-Deen *et al*., 2004)**.

Amonolyses of ester with 4-chloro-aniline in the presence of acid medium under fusion produced the N-(4-chlorophenyl)-7-hydroxycoumarin-3-yl carboxamide (comp-2) [scheme I]



Scheme (I): Synthesis of ethyl-7-hydroxycoumarin-3-ylester and N-(4-chlorophenyl)-7-hydroxycoumarin-3-yl carboxamide derivatives.

2.2.2. Cytotoxicity assay by 3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyltetrazolium bromide (MTT):

Exponentially growing cells from different cancer cell lines were trypsinized, counted and seeded at the appropriate densities (5000 cells/0.33 cm^2 well) into 96-well microtiter plates. Cells then were incubated in a humidified atmosphere at 37^oC for 24 hours. Then, cells were exposed to different concentrations of compounds (0.05, 0.5, 5, 50, and 500µg/ml) for 72 hours as illustrated in table (1). Then the viability of treated cells was determined using MTT technique as follow. Media were removed; cells were incubated with 200µl of 5% MTT solution /well (Sigma Aldrich, MO) and were allowed to metabolize the dye into a colored –insoluble formazan crystal for 2 hours. The remaining MTT solution were discarded from the wells and the formazan crystals were dissolved in 200 µl/well acidified isopropanol for 30 min, covered with aluminum foil with continuous shaking by using a MaxQ 2000 plate shaker (Thermo Fisher Scientific Inc, MI) at room temperature. Absorbance was measured at 570 nm by using a Stat Fax^R 4200 plate reader (Awareness Technology, Inc., FL). The cell viability were expressed as percentage of control and the concentration that induces 50% of maximum inhibition of cell proliferation (IC50) were determined using Graph Pad Prism version 5 software (Graph Pad software Inc,CA) **(Mosmann, 1983 and Scudiero *et al*., 1988)**.

3. RESULTS

Cytotoxicity: The in vitro cytotoxic activities of compounds: N-(P-chlorophenyl)-7-hydroxycoumarin-3-yl carboxamide (comp-1) and Ethyl 7-hydroxycoumarin-3-yl ester (comp-2) were showed in table (1) and figures (1-4).

Minimum inhibitory concentrations of synthesized compound N-(P-chlorophenyl)-7-hydroxycoumarin-3-yl carboxamide (comp-1) were found to be 12µg/ml, 9.7µg/ml, 18µg/ml and 14.4µg/ml against MCF-7, HEPG-2, HCT, PC-3 cell lines, respectively.

While, Minimum inhibitory concentrations of synthesized compound Ethyl 7-hydroxycoumarin-3-yl ester (comp-2) were found to be 67.5µg/ml, 87µg/ml, 218µg/ml and 91µg/ml against MCF-7, HEPG-2, HCT, PC-3 cell lines, respectively.

Table (1): Minimum inhibitory concentrations of synthesized compounds (comp-1 and comp-2) against MCF-7, HEPG-2, HCT and PC-3 cell line.

Variable	MCF-7	HEPG-2	HCT	PC-3
Comp-1	12	9.7	18	91
Comp-2	67.5	87	218	91

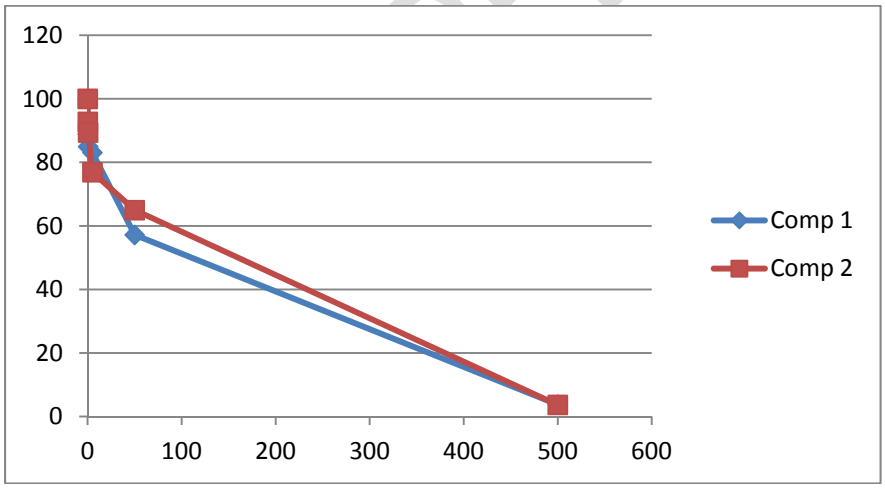


Fig. (1): Minimum inhibitory concentration of comp-1 and comp-2 against MCF-7 cell line

*IC50 of comp-1aganist MCF-7 is 12 µg/ml while comp-2 is 67.5 µg/ml

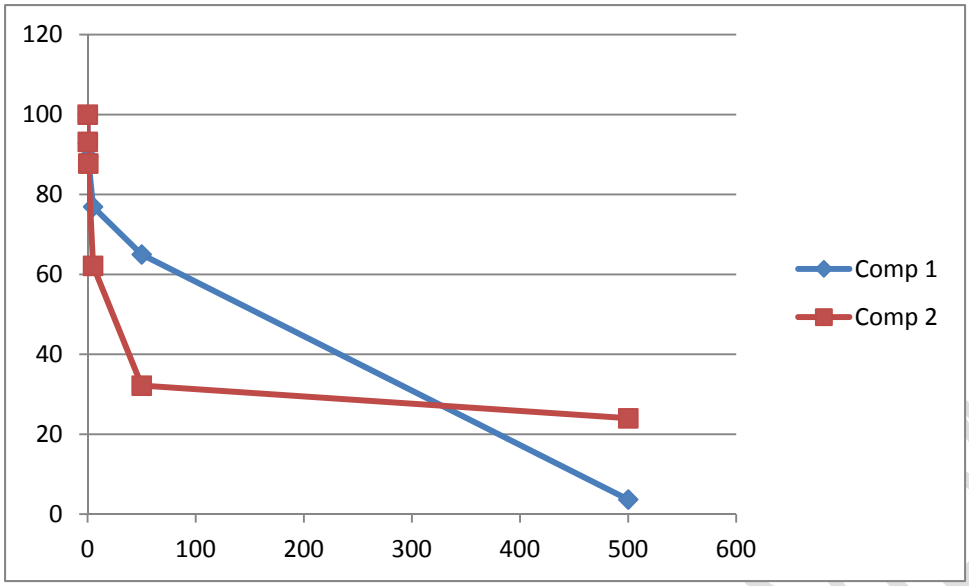


Fig. (2): Minimum inhibitory concentration of comp-1 and comp-2 against HEPG-2 cell line

*IC50 of comp-1 against HEPG-2 is 9.7 µg/ml while comp-2 is 87 µg/ml

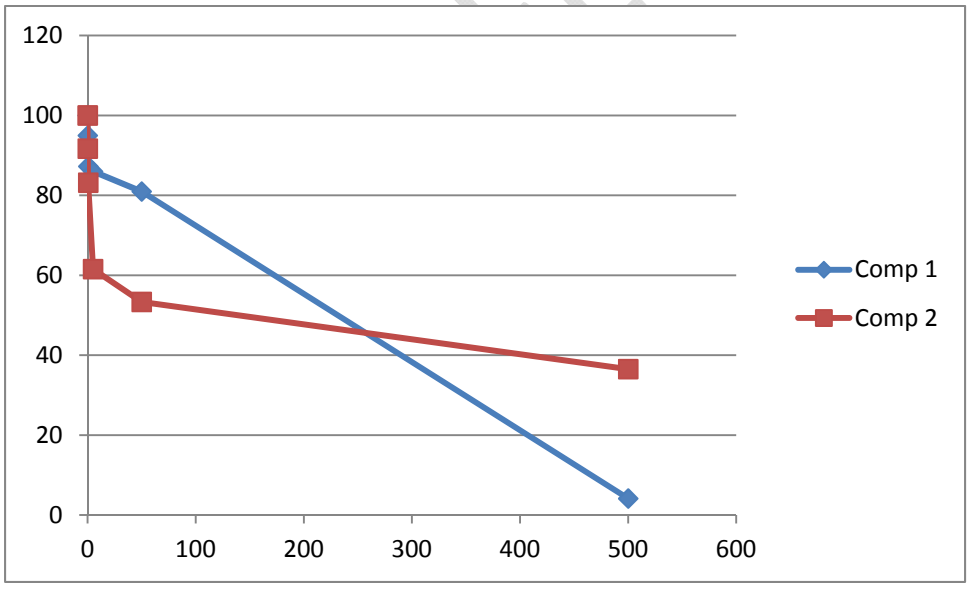


Fig. (3): Minimum inhibitory concentration of comp-1 and comp-2 against HCT cell line.

*IC50 of comp-1 against HCT is 18 µg/ml while comp-2 is 218 µg/ml

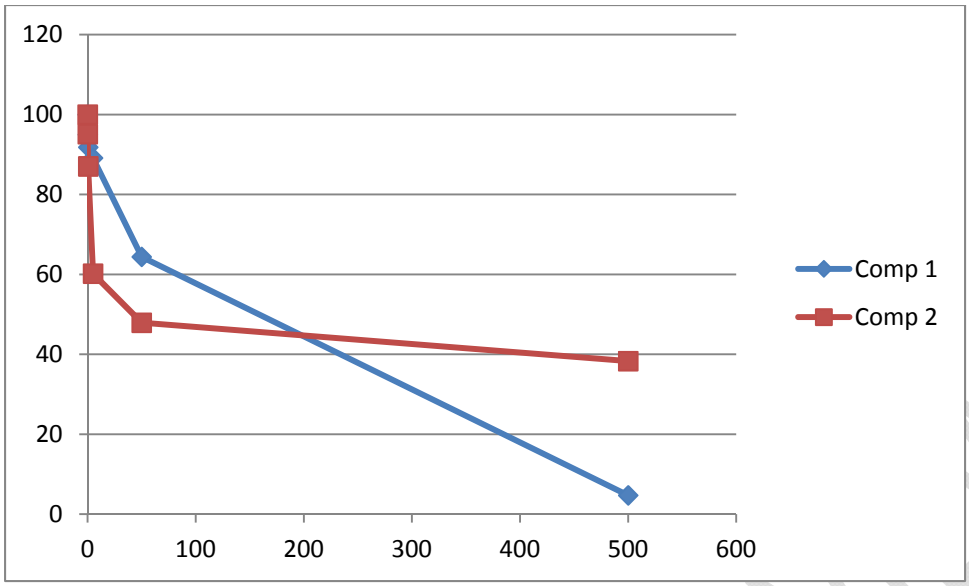


Fig. (4): Minimum inhibitory concentration of comp-1 and com-2 against PC-3 cell line

*IC50 of comp-1 against HCT is 91 µg/ml while comp-2 is 91 µg/ml

4. DISCUSSION

Cancer is now one of the world's most pressing health challenges. Research continues to deliver new and improved treatment options for thousands of people living with cancer **(ASC, 2016)**. Cancer have not been cured yet. It is estimated that by 2020 there will be 16 million new cancer cases every year (**Lingwood et al., 2008)**. The chemistry of heterocyclic compounds continues to be an explore field in the organic or Pharmaceutical chemistry. The Coumarin (benzopyran-2 one, or chromen-2-one) ring display interesting pharmacological properties has intrigued chemists and medicinal chemists for decades to explore the natural Coumarins or synthetic analogs for their applicability as drugs. Some new derivatives bearing coumarin ring including the furanocomarins (e.g., Imperatorin), pyranocoumarins (e.g., Seselin), and coumarin sulfamates (Coumates), have been found to be useful in photo-chemotherapy, antitumor and anti-HIV therapy (**Kostova *et al.*, 2006)**. All these findings encouraged us to explore the synthesis of coumarin derivatives and examine their activities as in vitro anti-cancer against some different cell lines such as [MCF-7(human breast cancer), HePG2 (Hepatocellular carcinoma), HCT (human colon cancer), PC3 (human prostate cancer)] to assess their cytotoxicity effects. The results indicated that N-(P-chlorophenyl)-7-hydroxycoumarin-3-yl carboxamide (comp-1) has cytotoxicity potency. N-(P-chlorophenyl)-7-hydroxycoumarin-3-yl carboxamide (comp-1) showed a very potent activity against MCF-7, HePG2, HCT, and PC3 with minimum inhibitory concentration [12, 9.7, 18, and 91 µg/ml, respectively] but Ethyl 7-hydroxycoumarin-3-yl ester (comp-2) showed low activity against MCF-7, HePG2, HCT, and PC3 than comp-1 with minimum inhibitory concentration [67.5, 87, 218, 91 µg/ml, respectively] compared with doxorubicin as reference drug. The most intriguing

biological activities of Coumarins is the notable effect of, some of the Coumarins against breast cancer, some Coumarins and their active metabolite 7-hydroxycoumarin analogs have shown sulfatase and aromatase inhibitory activities (**Momekov *et al*., 2006)**. Coumarin based selective estrogen receptor modulators (SERMs) and Coumarin estrogen conjugates have also been described as potential anti-breast cancer agents according some recently publications (**You *et al*., 2010)**. The natural form of coumarin itself has demonstrated an anti-tumor activity. Coumarin (known as 1, 2-benzopyrone), consisting of fused benzene and α-pyrone ring, is an important group of low molecular weight (**Fylaktakidou *et al*., 2004)** . This effect is probably linked to its metabolites (*e.g.* 7-hydroxycoumarin, 7-HC) transformed by cytochromes P450 **(Pelkonen *et al*., 2000)**. Recently, several groups have attempted to establish a structure activity relationship (SAR) between coumarins and their various anticancer properties **(Bruyere *et al*., 2011)**. The hydroxyl group on position C-7 seems to be pivotal for the anticancer activity **(Wu *et al*., 2009)**. Moreover, 7-HC and several of its derivatives inhibit proteins implicated in the cell cycle and overexpressed in many types of cancers, such as Cyclin D1 and Cdc25 **(Jimenez-Orozco *et al*., 2001) (Valente *et al*., 2001)**. Our results agreed with **Stanway *et al*.,(2006)**, who studied the growth-inhibitory cytostatic activity in human cancer cell line: MCF-7 breast carcinoma cells. They reported that, osthole "Coumarin derivatives" demonstrated some estrogenic activity by preventing the synthesis and action of estrogens (ER antagonists), and this indicated that, osthole has the potential to be a breast cancer treatment reagent. As **Kempen *et al***., who stated that, the inhibition capacity varied according to the substituent present in the 6-position of the coumarin, and according to the nature of the halogen atom in the 3-position of the phenyl ring. In general, (substitution by a halogen atom particularly, a chlorine or a bromine atom) in the 'meta' position of the phenyl ring relative to the ester oxygen atom of 2-oxo-2H-1-benzopyran- 3-carboxylate led to a better anti-tumor effect than that observed in the absence of any substituent **(Kempen *et al*., 2003) (El-behary *et al*., 2013) .** Our results agreed with **El-behary *et al*., 2013,** who studied the cytotoxicity of new coumarin derivatives: Potassium salt of 2-thioxo-4-hydroxycoumarin [3, 4-b] pyrimidine and 9-bromo-2-thioxo-4-hydroxycoumarin [3, 4-b] pyrimidine against some different cell lines such as [MCF-7(human breast cancer), HePG2 (Hepatocellular carcinoma), HCT (human colon cancer), PC3 (human prostate cancer)].

5. CONCLUSIONS

The in vitro cytotoxic activity for the compounds: N-(P-chlorophenyl)-7-hydroxycoumarin-3-yl carboxamide and Ethyl 7-hydroxycoumarin-3-yl ester (comp-1 & comd-2) against the human breast tumor cells (MCF-7), human hepatocellular cancer cells (HePG2), HCT16 (colon cancer), and PC3 (prostate cancer). Comp-1 exhibits minimum inhibitory concentration against all cell lines at higher doses than comp-2. On the basis of these results, comp-1 may be considered as attractive leads in the future development of potential anticancer agent more than comp-2.

6. REFERENCES

National Cancer Institute." What is cancer?" Cancer.gov. (2013).

ASC “American Society of Oncology”, (2016): Clinical Cancer Advances.

Faten A. Khorshid (2011): The cytotoxic effect of PM 701 and its fractions on cell proliferation of Breast cancer cells, MCF7. American Journal of Drug Discovery and development., 1(3): 200-208.

A.R Sherif (2010): Polysubstituted pyrazoles, part 6. Synthesis of some 1-(4-chlorophenyl)-4-hydroxy-1H-pyrazol-3-carbonyl derivatives linked to nitrogenous heterocyclic ring systems as potential antitumor agents. Bioorganic & Medicinal Chemistry., 18(7), 2767–2776.

J. Klenkar and M. Molnar (2015): Natural and synthetic coumarins as potential anticancer agents. Journal of Chemical and Pharmaceutical Research., 7(7): 1223-1238.

A . Lacy and R. O'Kennedy (2004): Studies on coumarins and coumarin-related compounds to determine their therapeutic role in the treatment of cancer. Curr Pharm Des., 10(30):3797–3811**.**

P. K. Jain and Himanshu Joshi (2012): Coumarin: Chemical and Pharmacological Profile. Journal of Applied Pharmaceutical Science., 2(6): 236-240.

S-J. Lee, U-S. Lee, W-J. Kim and S-K. Moon (2011): Inhibitory effect of esculetin on migration, invasion and matrix metalloproteinase-9 expression in TNF-alpha-induced vascular smooth muscle cells. Molecular Medicine Reports., 4:337-341.

LW. Nitiema, A. Savadogo, J. Simpore, D. Dianou and A.S. Traore (2012): In vitro Antimicrobial Activity of Some Phenolic Compounds (Coumarin and Quercetin) Against Gastroenteritis Bacterial Strains. International Journal of Microbiological Research., 3 (3): 183-187.

B. Xu, L. Wang, L. Gonzalez-Molleda, Y. Wang, J. Xu *et al* (2014): Antiviral Activity of (+)-Rutamarin against Kaposi's Sarcoma-Associated Herpesvirus by Inhibition of the Catalytic Activity of Human Topoisomerase II. Antimicrob Agents Chemother., 58(1), 563-573**.**

I. Kostova, S. Bhatia, P. Grigorov, S. Balkansky, V.S. Pramar, *et al* (2011): Coumarins as antioxidants. Curr Med Chem., 18(25), 3929-3951.

P. Anand, B. Singh and N. Singh (2012): A review on coumarins as acetylcholinesterase inhibitors for Alzheimer's disease. Bioorg Med Chem., 20(3), 1175-1180.

X.Y. Huang, Z.J. Shan, H.L. Zhai, L. Su and XY. Zhang (2011): Study on the anticancer activity of coumarin derivatives by molecular modeling. Chem Biol Drug Des.,78(4), 651-658.

A. Sánchez-Recillas, G. Navarrete-Vázquez, S. Hidalgo-Figueroa, MY. Rios, M. Ibarra-Barajas, *et al* (2014): Semisynthesis, ex vivo evaluation, and SAR studies of coumarin derivatives as potential antiasthmatic drugs. European Journal of Medicinal Chemistry.,77, 400-408.

K.V. Sashidhara, R.K. Modukuri, S. Singh, K.B. Rao, G.A. Teja, et al (2015): Design and synthesis of new series of coumarin- amino pyran derivatives possessing potential anti- depressant- like activity. Bioorganic & Medicinal Chemistry Letters., 25, 337-341.

E. Kudo, M. Taura, K. Matsuda, M. Shimamoto, R. Kariya, *et al* (2015): Inhibition of HIV-1 entry by the tricyclic coumarin GUT-70 through the modification of membrane fluidity. Biochem Biophys Res Commun., 457(3):288-94.

A. Manvar, A. Bavishi, A. Radadiya, J. Patel, V. Vora, *et al* (2011): Diversity oriented design of various hydrazides and their in vitro evaluation against Mycobacterium tuberculosis H37Rv strains. Bioorganic & Medicinal Chemistry Letters., 21(16), 4728-4731.

J.Y. Yeh, M.S. Coumar, J.T. Horng, H.Y. Shiao, F.M. Kuo, *et al* (2010): Anti-influenza drug discovery: structure activity relationship and mechanistic insight into novel angelicin derivatives. J. Med. Chem., 53(4), 1519–1533.

M. Asif (2015): Pharmacologically potentials of different substituted coumarin derivatives. Chemistry International., 1(1), 1-11.

J. Wang, M.L. Lu, H.L. Dai, S.P. Zhang, H.X. Wang, *et al* (2015): Esculetin, a coumarin derivative, exerts *in vitro* and *in vivo* antiproliferative activity against hepatocellular carcinoma by initiating a mitochondrial-dependent apoptosis pathway. Braz. J. Med. Biol. Res., 48(3), 245-253.

K.M. Amin, A.M. Eissa, S.M. Abou-Seri, F.M. Awadallah and G.S. Hassan (2013): Synthesis and biological evaluation of novel coumarin-pyrazoline hybrids endowed with phenylsulfonyl moiety as antitumor agents. Eur. J. Med. Chem., 60, 187-198.

T. Nasr, S. Bondock and M. Youns (2014): Anticancer activity of new coumarin substituted hydrazide-hydrazone derivatives. Eur. J. Med. Chem., 76, 539-548.

K. Benci, L. Mandić, T. Suhina, M. Sedić, M. Klobučar *et al* (2012): Novel Coumarin Derivatives Containing 1,2,4-Triazole, 4,5-Dicyanoimidazole and Purine Moieties: Synthesis and Evaluation of Their Cytostatic Activity. Molecules. 17(9), 11010-11025.

M.E. Marshall, K. Kervin, C. Benefield, A. Umerani, S. Albainy-Jenei et al (1994): Growth-inhibitory effects of coumarin (1,2-benzopyrone) and 7-hydroxycoumarin on human malignant cell lines in vitro. J Cancer Res Clin Oncol., 120(1), 3-10.

J.L. Mohler ,L.G. Gomella , E.D. Crawford, L.M. Glode, C.D. Zippe et al (1992): Phase II evaluation of coumarin (1,2-benzopyrone) in metastatic prostatic carcinoma. Prostate., 20:123–131.

R.D. Thornes, L. Daly, G. Lynch, B. Breslin, H. Browne, et al (1994): Treatment with coumarin to prevent or delay recurrence of malignant melanoma. Journal of Cancer Research and Clinical Oncology., 120(1), 32–S34.

M.K. Marshall , K. Butler and A. Fried (1991): Phase I evaluation of coumarin (1,2-benzopyrone) and cimetidine in patients with advanced malignancies. Mol Biother., 3:170–178.

S. Mirunalini, K. Deepalakshmi and J. Manimozhi (2014): Antiproliferative effect of coumarin by modulating oxidant/ antioxidant status and inducing apoptosis in Hep2 cells. *Biomed. Aging Pathol*., 4(2), 131-135.

T. Mosmann (1983): Rapid colorimetric assay for cellular growth and survival : application to proliferation and cytotoxicity assays. J. Immunol. Methods., 65(1-2):55-63.

D.A. Scudiero, *et al*. (1988): Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using a human and other tumor cell lines. Cancer Res., 48(17): 4827-33.

M.A. Musa, J.S. Cooperwood, M.O. Khan (2008): A review of coumarin derivatives in pharmacotherapy of breast cancer. Curr. Med. Chem., 15, 2664.

R. J. Lingwood, P. Boyle, A. Milburn, T. Ngoma, J. Arbuthnott, *et al* (2008): The challenge of cancer control in Africa. Nat Rev. Cancer., 8(5): 398-403.

I. Kostova, S. Raleva, P. Genova, R. Argirova (2006): Structure-Activity Relationships of Synthetic Coumarins as HIV-1 Inhibitors . Bioinorganic. Chem. Appl., 68274, 1-9.

G. Momekov, I. Kostova, T. Tzanova and M. Karaivanova (2006): Synthesis, Characterization and Cytotoxic Activity of New Lanthanum (III) Complexes of Bis Coumarins Irena. Bioinorganic. Chem. Appl., 25651, 1.

L. You , R. An , X. Wang and Y. Li (2010): Discovery of novel osthole derivatives as potential anti-breast cancer treatment. Bioorganic & Medicinal Chemistry Letters., 20, 7426–7428.

K.C. Fylaktakidou, D.J. Hadjipavlou-Litina, K.E. Litinas, D.N. Nicolaides (2004): Natural and synthetic coumarin derivatives with anti-inflammatory/antioxidant activities. Curr Pharm Des., 10:3813–33.

S.J. Stanway, A. Purohit, L.W. Woo, S. Sufi, D. Vigushin *et al*. (2006): Phase I study of STX 64 (667 Coumate) in breast cancer patients: the first study of a steroid sulfatase inhibitor. Clin. Cancer Res., 12, 1585.

O. Pelkonen, A. Rautio, M. Pasanen and H. Raunio (2000): CYP2A6: A human coumarin 7-hydroxylase. *Toxicology., 144*, 139-147.

C. Bruyere, S. Genovese, B. Lallemand, A. Ionescu-Motatu, M. Curini, *et al (2011):* Growth inhibitory activities of oxyprenylated and non-prenylated naturally occurring phenylpropanoids in cancer cell lines. *Bioorg. Med. Chem. Lett., 21*, 4174-4179.

M. Curini, G. Cravotto, F. Epifano and G. Giannone (2006): Chemistry and biological activity of natural and synthetic prenyloxycoumarins. *Curr. Med. Chem., 13*, 199-222.

L. Wu, X. Wang, W. Xu, F. Farzaneh and R. Xu (2009): The structure and pharmacological functions of coumarins and their derivatives. *Curr. Med. Chem., 16*, 4236-4260.

F.A. Jimenez-Orozco, J.S. Lopez-Gonzalez, A. Nieto-Rodriguez, M.A. Velasco-Velazquez, J.A. Molina-Guarneros *et al* (2001): Decrease of cyclin D1 in the human lung adenocarcinoma cell line A-427 by 7-hydroxycoumarin. *Lung Cancer., 34*, 185-194.

S. Valente, E. Bana, E. Viry, D. Bagrel, G. Kirsch (2010): Synthesis and biological evaluation of novel coumarin-based inhibitors of Cdc25 phosphatases. *Bioorg. Med. Chem. Lett.*, *20*, 5827- 5830.

F. Z. Mohamed, I. M. EL-Deen, M.M. El-behary and K.T. Akaber (2013): Potassium salt of 2-thioxo-4-hydroxycoumarin [3, 4-b pyrimidine and 9-bromo-2-thioxo-4-hydroxycoumarin -4 ,3[b] pyrimidine inhibits tumor growth in vitro and in vivo. INDIAN JOURNAL OF APPLIED RESEARCH., 3(6), 481- 485.

N.D. Vani, H.K. Jung, H. Ki-Cheol, G.Y. Eun, C. Hyunach *et al* (2010): Novel 6-N-arylcarboxamidopyrazolo[4,3-d]pyrimidin-7-one derivatives as potential anti-cancer agents. Bioorganic & Medicinal Chemistry Letters., 20: 1630-1633.

I. Kempen, D. Papapostolou , N. Thierry, L. Pochet, S. Counerotte, *et al* (2003): 3-Bromophenyl-6-acetoxymethyl-2-oxo-2H-1-benzopyran-3carboxylate Inhibits cancer cell invasion in vitro and tumor growth in vivo. British Journal of Cancer. 88: 1111-1118.