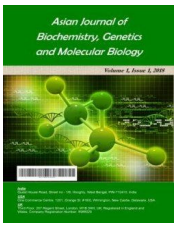


Asian Journal of Biochemistry, Genetics and Molecular Biology

2(1): 1-11, 2019; Article no.AJBGMB.49084

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

A. S. Tmamm[1*], F. Z. Mohammed[2] and I. M. El-Deen[3]

[1]Department of Chemistry, Biochemistry Branch, Faculty of Science, Port Said University, Port Said, Egypt.
[2]Department of Chemistry, Biochemistry Branch, Faculty of Science, Zagazig University, Zagazig, Egypt.
[3]Department of Chemistry, Faculty of Science, Port Said University, Port Said, Egypt.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJBGMB/2019/v2i130052
Editor(s):
(1) Dr. Gul Ozcan, Professor, Department of Biology, Faculty of Science, University of Istanbul, Turkey.
(2) Dr. Mohammed Rachidi, Director of Research, Molecular Genetics of Human Diseases (MGHD), French Polynesia, University Paris Denis Diderot, Paris, France.
Reviewers:
(1) Manojit Pal, DRILS, India.
(2) E. Siva Rami Reddy, Tantia University, India.
Complete Peer review History: http://www.sdiarticle3.com/review-history/49084

Original Research Article

Received 03 March 2019
Accepted 14 May 2019
Published 20 May 2019

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 (comp-3) and Ethyl 7-hydroxycoumarin-3-yl ester (comp-2) 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-2) was prepared via cyclocon-densation 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-3).

**Corresponding author: E-mail: salehasmaa789@gmail.com;*

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 (comp-3) is better than Ethyl 7-hydroxycoumarin-3-yl ester (comp-2) 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 failure of tissue growth regulation when the genes that regulate cell growth and differentiation are altered. Most cancers have multiple causes, only a small minority of cancer is due to inherited genetic mutations whereas the vast majority is non-hereditary epigenetic mutations that are caused by various agents (environmental factors, physical factors and hormones). Thus, although there are some genetic predispositions in a small fraction of cancers, the major fraction is due to a set of new genetic mutations (called "epigenetic" mutations) [1]. Therefore the search for potent, safe and selective anticancer compounds is a crucial aspect of modern cancer research [2]. 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 [3].

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 [4].

Coumarins belong to benzopyrone chemical class, more precisely benzo-α-pyrones, where benzene ring is fused to pyrone ring [5]. 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 [6].

Coumarins are proven to possess a wide range of biological activities, anti-influenza [7], anti-inflammatory [8], antioxidant [9], antitumor [10], antituberculosis [11], antimicrobial [12], antinociceptive, anti- Alzheimer [13], antiasthmatic [14], antiviral [15], anti-HIV [16], antidepressant [17], antihyperlipidemic [18].

Antitumor activity of natural and synthetic coumarin derivatives have been extensively explored by many researchers [19] 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 [20,21].

Coumarin derivatives can possess not only cytostatic, but cytotoxic properties as well [22]. Marshall et al. [23] 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 [24], malignant melanoma [25].

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 [26].

Our study aims to evaluate the cytotoxicity properties of recently developed synthetic coumarin derivatives: N-(P-chlorophenyl)-7-hydroxycoumarin-3-yl carboxamide (comp-3) and Ethyl 7-hydroxycoumarin-3-yl ester (comp-2) 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 100 mg/ 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 37ºC.

2.2 Methods

2.2.1 Chemistry

The ethyl-7-hydroxycoumarin-3-ylester (comp-2) was prepared via cyclocondensation of 2, 4-dihydroxybenzaldhyde (1) with diethylmalonate in the presence of piperidine under fusion according to a literature method [27].

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-3) [Scheme I].

2.2.2 Synthesis

All reagents were used as purchased from commercial supplies without further purification.

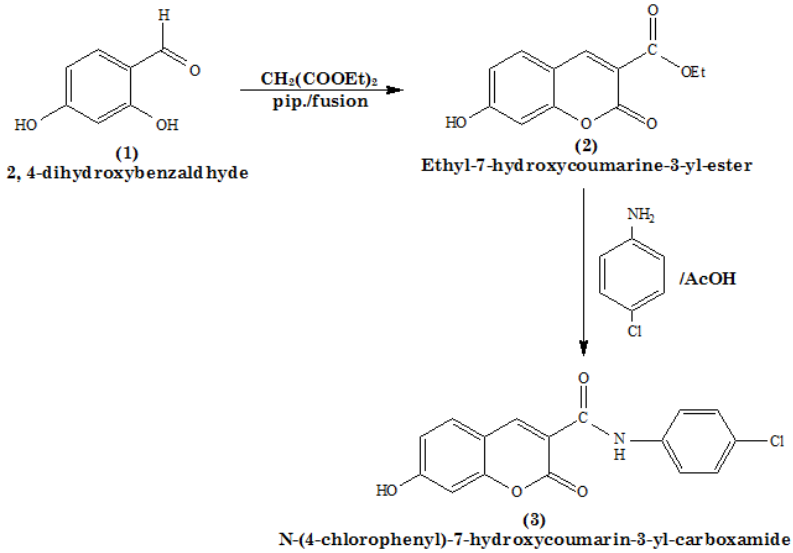
Melting points were determined by using open capillary tubes and were uncorrected.

The purity of the compound was determined through elemental analysis. Elemental data of C, H and N was found in accordance with ± 0.3% of the theoretical value, respectively as determined by PerkinElmer CHN elemental analyzer.

Using KBr pellets, IR spectrum were obtained with FT-IR spectrometer. ^{1}H-NMR spectra in DMSO-d_6 solutions were respectively recorded at 400 MHz with Bucker 400 ultra –shield TM NMR spectrometer (400 MH_Z) using TMS as internal standard.

Ethyl 7-hydroxycoumarin-3-ylester (1)

A mixture of 2, 4-dihydroxybenzaldehyde (1, 0.01 mole), diethylmalonate (0.01 mole), and piperidine was fused on a hot- plate for 3-4 min, then added ethanol (30 ml).



Scheme I. Synthesis of ethyl-7-hydroxycoumarin-3-ylester (comp-2) and N-(4-chlorophenyl)-7-hydroxycoumarin-3-yl carboxamide (comp-3) derivatives

The reaction mixture was heated under reflux for 2 hour, then cooled and acidified with diluted hydrochloric acid (2%). The solid product was filtered off, dried, and crystallized from ethanol to give 2 as pale yellow crystals, yield 76%, and m.p 165ºC.

IR (KBr): 3416-2815(br-oH), 1764-1722(C=O of ester and pyranone ring), 1610-1585(C=C), 1125-1095(C-O) cm^{-1}. ^{1}H-NMR(DMSO-d_6): δ 1.32(m,3H,CH_3), 4.41(g,2H,OCH_2),7.40-8.01 (m,3H,Ar-H), 8.53(δ,1H,H-4 of pyranone ring) , 10.7(br-S,1H,OH) ppm. Anol.calcd for $C_{12}H_{10}O_5$ (234): C, 61.54; H, 4.27. Found: C, 61.52; H, 4.17.

N-(P-chlorophenyl)-7-hydroxycoumarin-3-ylcarboxamide (2)

A mixture of ester (2, 0.01 mole) and p-chloroaniline (0.01 mole) in acetic acid (25ml) was heated under reflux for 4 hour. The reaction mixture was cooled and poured into ice –water with stirring. The resulting solid was collected by filtration ,washed with water, dried and recrystallized from ethanol to give 3 as yellow crystals , yield 71% ,mp.>300 ^oC.

IR (KBr): 3430-2981 (br.OH), 3256(NH), 1726-1708(C=O of pyranone and carboxamide), 1615-158(C=C), 1093-1065(C-O) cm^{-1}. ^{1}H-NMR(DMSO-d_6):δ 6.76-7.88(m,7H,Ar-H),8.69(S,1H,H-4 of pyranone ring), 8.87 (S,1H,NH),10.71(S,1H,OH)ppm. Anol.calcd for $C_{16}H_{10}NClO_4$ (315): C, 60.95; H, 3.17; N4.44.Found: C, 60.73; H3.08; N, 4.11.

2.2.3 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) [28] and [29].

3. RESULTS

Cytotoxicity: The *in vitro* cytotoxic activities of compounds

N-(P-chlorophenyl)-7-hydroxycoumarin-3-yl carboxamide (comp-3) and Ethyl 7-hydroxycoumarin-3-yl ester (comp-2) were showed in Table 1 and Figs. 5-8.

Minimum inhibitory concentrations of synthesized compound N-(P-chlorophenyl)-7-hydroxycoumarin-3-yl carboxamide (comp-3) were found to be 12 µg/ml, 9.7 µg/ml, 18 µg/ml and 14.4 µg/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 67.5 µg/ml, 87 µg/ml, 218 µg/ml and 91 µg/ml against MCF-7, HEPG-2, HCT, PC-3 cell lines, respectively.

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

Variable	MCF-7 µg/ml	HEPG-2 µg/ml	HCT µg/ml	PC-3 µg/ml
Comp-3	12	9.7	18	14.4
Comp-2	67.5	87	218	91
doxorubicin	56	78	160	80

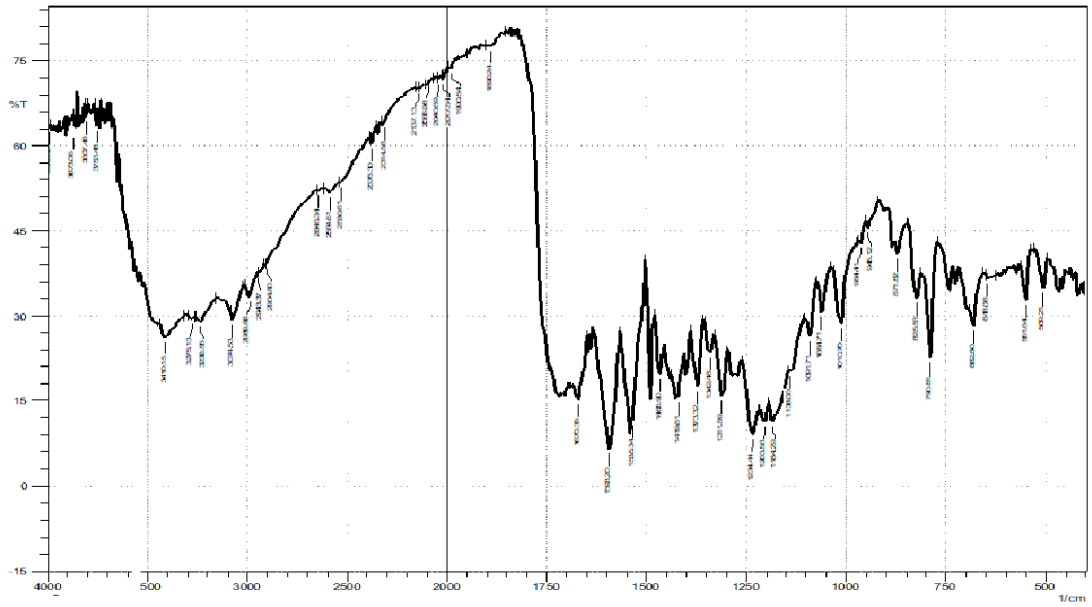


Fig. 1. IR spectroscopy of ester (comp-2)

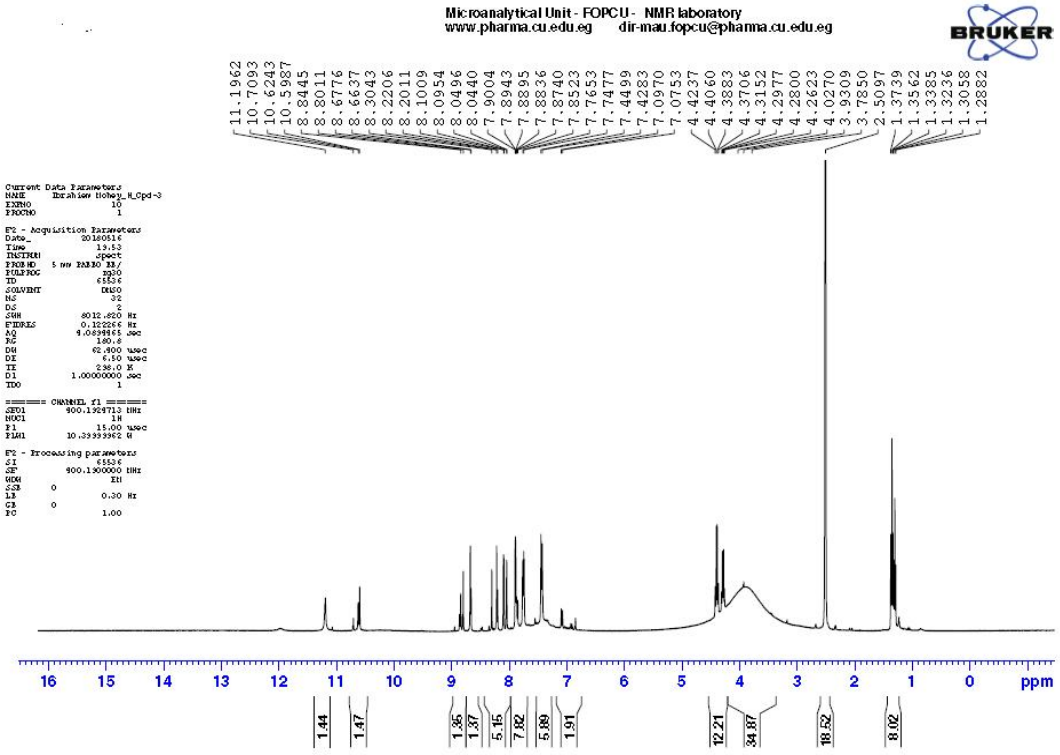


Fig. 2. HNMR spectroscopy for ester (comp-2)

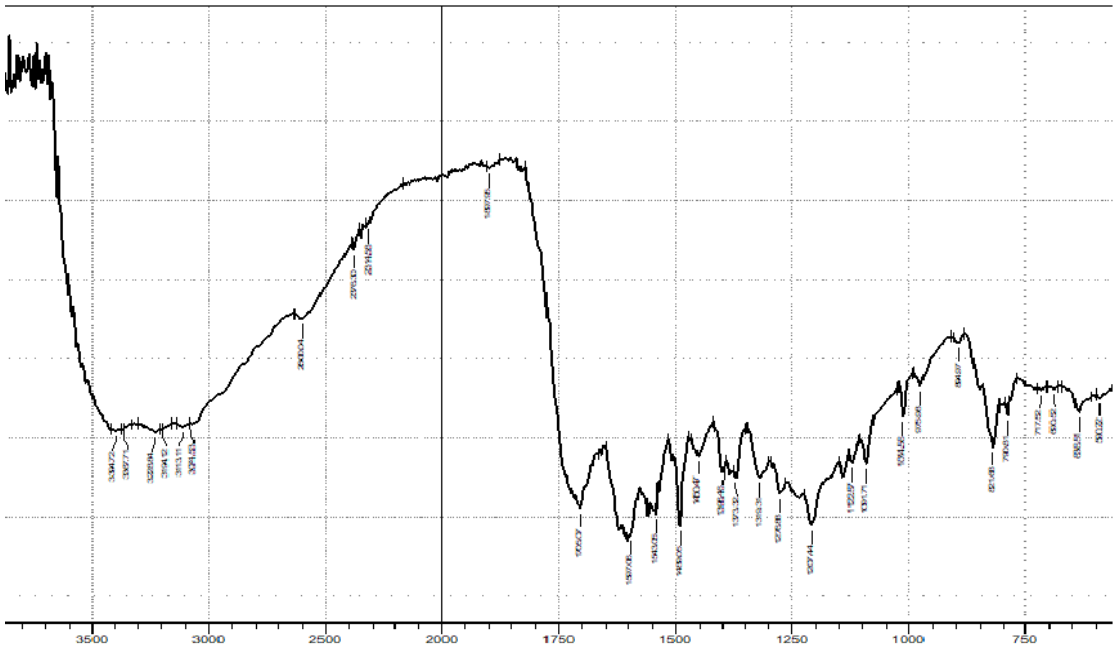


Fig. 3. IR spectroscopy of carboxamide (comp-3)

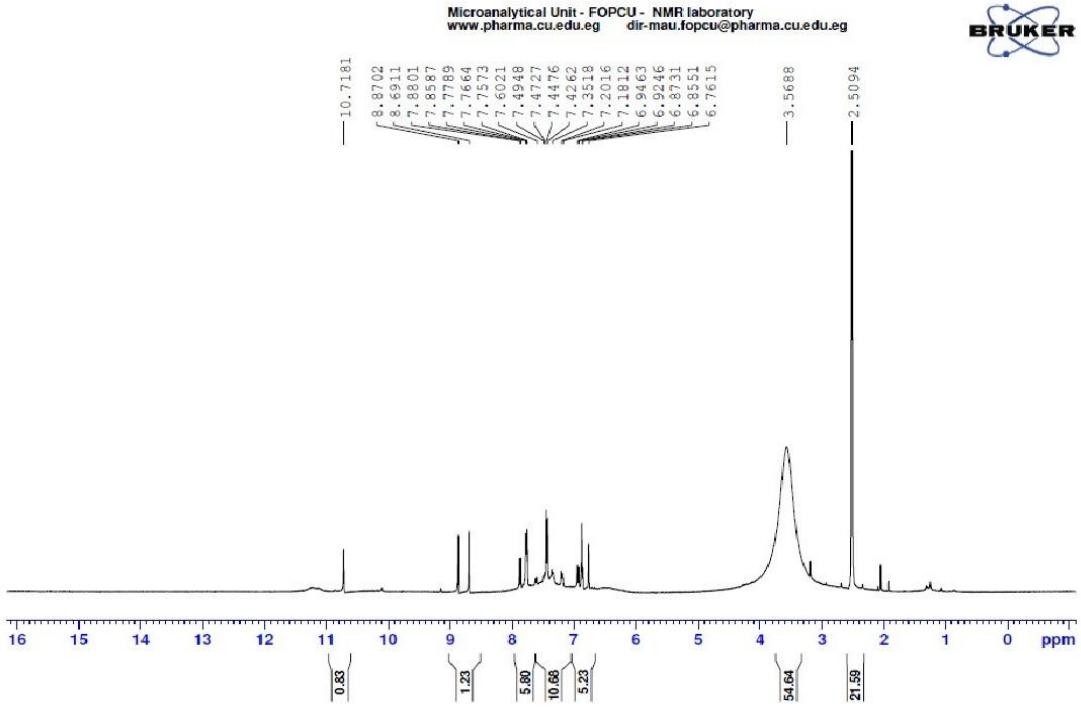


Fig. 4. HNMR spectroscopy of carboxamide (comp-3)

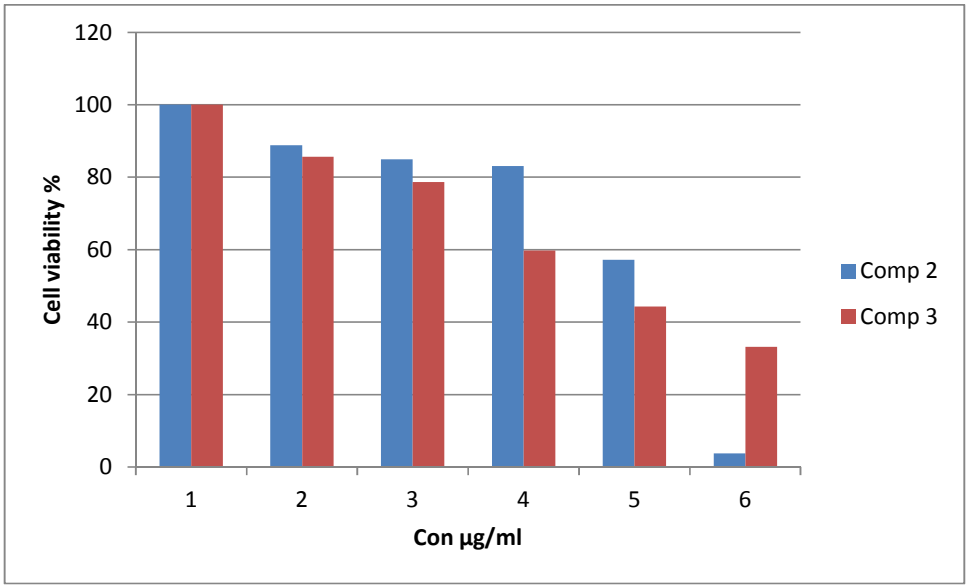


Fig. 5. Minimum inhibitory concentration of comp-2 and comp-3 against MCF-7 cell line
**IC50 of comp-3 against MCF-7 is 12 µg/ml while comp-2 is 67.5 µg/ml*

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 [30]. Cancer has not been cured yet. It is estimated that by 2020 there will be 16 million new cancer cases every year [31]. 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 [32]. 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-3) has cytotoxicity potency. N-(P-chlorophenyl)-7-hydroxycoumarin-3-yl carboxamide (comp-3) showed a very potent activity against MCF-7, HePG2, HCT, and PC3 with minimum inhibitory concentration [12, 9.7, 18, and 14.4 µg/ml, respectively] but Ethyl 7-hydroxycoumarin-3-yl ester (comp-2) showed low activity against MCF-7, HePG2, HCT, and PC3 than comp-3 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 [33]. 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 [34]. 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 [35]. This effect is probably linked to its metabolites (*e.g.* 7- hydroxyl-coumarin, 7-HC) transformed by cytochromes P450 [36]. Recently, several groups have attempted to establish a structure activity relationship (SAR) between coumarins and their various anticancer properties [37]. The hydroxyl group on position C-7 seems to be pivotal for the anticancer activity [38]. 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 [39,40]. Our results agreed with Stanway et al. [41], 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. [42], 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 [42,43]. 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)].

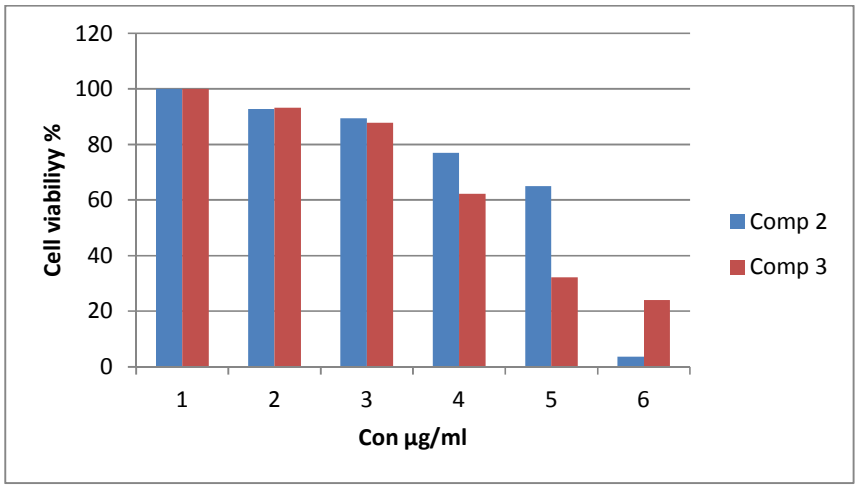


Fig. 6. Minimum inhibitory concentration of comp-2 and comp-3 against HEPG-2 cell line
**IC50 of comp-3 against HEPG-2 is 9.7 µg/ml while comp-2 is 87 µg/ml*

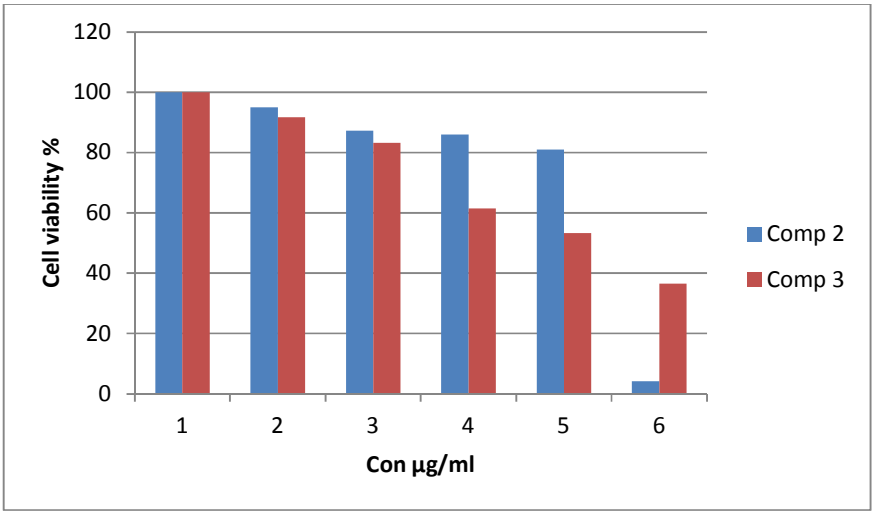


Fig. 7. Minimum inhibitory concentration of comp-2 and comp-3 against HCT cell line
**IC50 of comp-3 against HCT is 18 µg/ml while comp-2 is 218 µg/ml*

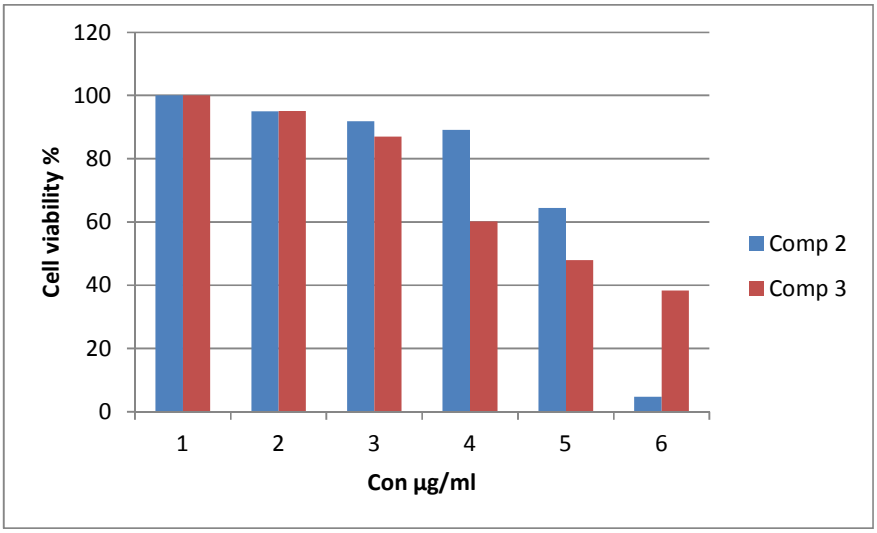


Fig. 8. Minimum inhibitory concentration of comp-2 and comp-3 against PC-3 cell line
**IC50 of comp-3 against HCT is 14.4 µg/ml while comp-2 is 91 µg/ml*

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-2 & comd-3) against the human breast tumor cells (MCF-7), human hepatocellular cancer cells (HePG2), HCT16 (colon cancer), and PC3 (prostate cancer). Comp-3 exhibits minimum inhibitory concentration against all cell lines at higher doses than comp-2. On the basis of these results, comp-3 may be considered as attractive leads in the future development of potential anticancer agent more than comp-2.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Alain LF. Genetics, Epigenetics and Cancer. Canc Therapy & Oncol Int J. 2017;4(2):555-634.
2. Vani ND, Jung HK, Ki-Cheol H, Eun GY, Hyunach C, AN Pae, et al. Novel 6-N-arylcarboxamidopyrazolo[4,3-d]pyrimidin-7-one derivatives as potential anti-cancer agents. Bioorganic & Medicinal Chemistry Letters. 2010;20:1630-1633.
3. Sherif AR. 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. 2010;18(7):2767–2776.
4. Klenkar J, Molnar M. Natural and synthetic coumarins as potential anticancer agents. Journal of Chemical and Pharmaceutical Research. 2015;7(7):1223-1238.
5. Lacy A, O'Kennedy R. Studies on coumarins and coumarin-related compounds to determine their therapeutic role in the treatment of cancer. Curr Pharm Des. 2004;10(30):3797–3811.
6. Jain PK, Himanshu Joshi. Coumarin: Chemical and pharmacological profile. Journal of Applied Pharmaceutical Science. 2015;2(6):236-240.
7. Yeh JY, Coumar MS, Horng JT, Shiao HY, Kuo FM, Lee HL, et al. Anti-influenza drug discovery: Structure activity relationship and mechanistic insight into novel angelicin derivatives. J. Med. Chem. 2010;53(4):1519–1533.
8. Lee SJ, Lee US, Kim WJ, Moon SK. Inhibitory effect of esculetin on migration, invasion and matrix metalloproteinase-9 expression in TNF-alpha-induced vascular smooth muscle cells. Molecular Medicine Reports. 2011;4:337-341.
9. Kostova I, Bhatia S, Grigorov P, Balkansky S, Pramar VS, Prasad AK, et al. Coumarins as antioxidants. Curr Med Chem. 2011;18(25):3929-3951.
10. Huang XY, Shan ZJ, Zhai HL, Su L, Zhang XY. Study on the anticancer activity of

coumarin derivatives by molecular modeling. Chem Biol Drug Des. 2011;78(4):651-658.
11. Manvar A, Bavishi A, Radadiya A, Patel J, Vora V, Dondia N, et al. Diversity oriented design of various hydrazides and their in vitro evaluation against Mycobacterium tuberculosis H37Rv strains. Bioorganic & Medicinal Chemistry Letters. 2011;21(16):4728-4731.
12. Nitiema LW, Savadogo A, Simpore J, Dianou D, Traore AS. *In vitro* antimicrobial activity of some phenolic compounds (Coumarin and Quercetin) against gastroenteritis bacterial strains. International Journal of Microbiological Research. 2012;3(3):183-18.
13. Anand P, Singh B, Singh N. A review on coumarins as acetylcholinesterase inhibitors for Alzheimer's disease. Bioorg Med Chem. 2012;20(3):1175-1180.
14. Sánchez-Recillas A, Navarrete-Vázquez G, Hidalgo-Figueroa S, Rios MY, Ibarra-Barajas M, Estrada-Soto S. Semisynthesis, *ex vivo* evaluation, and SAR studies of coumarin derivatives as potential antiasthmatic drugs. European Journal of Medicinal Chemistry. 2014;77:400-408.
15. Xu B, Wang L, Gonzalez-Molleda L, Wang Y, Xu J, Yuan Y. Antiviral activity of (+)-rutamarin against kaposi's sarcoma-associated herpesvirus by inhibition of the catalytic activity of human topoisomerase II. Antimicrob Agents Chemother. 2014;58(1):563-573.
16. Kudo E, Taura M, Matsuda K, Shimamoto M, Kariya R, Goto H, et al. Inhibition of HIV-1 entry by the tricyclic coumarin GUT-70 through the modification of membrane fluidity. Biochem Biophys Res Commun. 2015;457(3):288-94.
17. Sashidhara KV, Modukuri RK, Singh S, Rao KB, Teja GA, Gupta S, et al. Design and synthesis of new series of coumarin-amino pyran derivatives possessing potential anti- depressant- like activity. Bioorganic & Medicinal Chemistry Letters. 2015;25:337-341.
18. Asif M. Pharmacologically potentials of different substituted coumarin derivatives. Chemistry International. 2015;1(1):1-11.
19. Wang J, Lu ML, Dai HL, Zhang SP, Wang HX, Wei N, et al. 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. 2015;48(3):245-253.
20. Amin KM, Eissa AM, Abou-Seri SM, Awadallah FM, Hassan GS. Synthesis and biological evaluation of novel coumarin-pyrazoline hybrids endowed with phenylsulfonyl moiety as antitumor agents. Eur. J. Med. Chem. 2013;60:187-198.
21. Nasr T, Bondock S, Youns M. Anticancer activity of new coumarin substituted hydrazide-hydrazone derivatives. Eur. J. Med. Chem. 2014;76:539-548.
22. Benci K, Mandić L, Suhina T, Sedić M, Klobučar M, et al. Novel coumarin derivatives containing 1,2,4-Triazole, 4,5-dicyanoimidazole and purine moieties: Synthesis and evaluation of their cytostatic activity. Molecules. 2012;17(9):11010-11025.
23. Marshall ME, Kervin K, Benefield C, Umerani A, Albainy-Jenei S, et al. Growth-inhibitory effects of coumarin (1,2-benzopyrone) and 7-hydroxycoumarin on human malignant cell lines *in vitro*. J Cancer Res Clin Oncol. 1994;120(1):3-10.
24. Mohler JL, Gomella LG, Crawford ED, Glode LM, Zippe CD, et al. Phase II evaluation of coumarin (1,2-benzopyrone) in metastatic prostatic carcinoma. Prostate. 1992;20:123–131.
25. Thornes RD, Daly L, Lynch G, Breslin B, Browne H, et al. Treatment with coumarin to prevent or delay recurrence of malignant melanoma. Journal of Cancer Research and Clinical Oncology. 1994;120(1):32–S34.
26. Mirunalini S, Deepalakshmi K, Manimozhi J. Antiproliferative effect of coumarin by modulating oxidant/ antioxidant status and inducing apoptosis in Hep2 cells. Biomed. Aging Pathol. 2014; 4(2):131-135.
27. El-Deen IM, Ibrahim Hk. Synthesis and mass spectra of some new 3-substituted coumarin derivatives. Chem. Pap. 2004;58(3):200.
28. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods. 1993;65(1-2):55-63.
29. Scudiero DA, et al. 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. 1988;48(17):4827-33.
30. ASC. American Society of Oncology. Clinical Cancer Advances; 2016.

31. Lingwood RJ, Boyle P, Milburn A, Ngoma T, Arbuthnott J, McCaffrey R, et al. The challenge of cancer control in Africa. Nat Rev. Cancer. 2008;8(5):398-403.
32. Kostova I, Raleva S, Genova P, Argirova R. Structure-activity relationships of synthetic coumarins as HIV-1 inhibitors . Bioinorganic. Chem. Appl. 2006;68274:1-9.
33. Momekov G, Kostova I, Tzanova T, Karaivanova M. Synthesis, characterization and cytotoxic activity of new lanthanum (III) complexes of bis Coumarins Irena. Bioinorganic. Chem. Appl. 2006;25651:1.
34. You L, An R, Wang X, Li Y. Discovery of novel osthole derivatives as potential anti-breast cancer treatment. Bioorganic & Medicinal Chemistry Letters. 2010;20: 7426–7428.
35. Fylaktakidou KC, Hadjipavlou-Litina DJ, Litinas KE, Nicolaides DN. Natural and synthetic coumarin derivatives with anti-inflammatory/antioxidant activities. Curr Pharm Des. 2004;10:3813–33.
36. Pelkonen O, Rautio A, Pasanen M, Raunio H. CYP2A6: A human coumarin 7-hydroxylase. Toxicology. 2000;144:139-147.
37. Bruyere C, Genovese S, Lallemand B, Ionescu-Motatu A, Curini M, et al. Growth inhibitory activities of oxyprenylated and non-prenylated naturally occurring phenylpropanoids in cancer cell lines. Bioorg. Med. Chem. Lett. 2011;21:4174-4179.
38. Wu L, Wang X, Xu W, Farzaneh F, Xu R. The structure and pharmacological functions of coumarins and their derivatives. Curr. Med. Chem. 2009;16:4236-4260.
39. Jimenez-Orozco FA, Lopez-Gonzalez JS, Nieto-Rodriguez A, Velasco-Velazquez MA, Molina-Guarneros JA, et al. Decrease of cyclin D1 in the human lung adenocarcinoma cell line A-427 by 7-hydroxycoumarin. Lung Cancer. 2001;34: 185-194.
40. Valente S, Bana E, Viry E, Bagrel D, Kirsch G. Synthesis and biological evaluation of novel coumarin-based inhibitors of Cdc25 phosphatases. Bioorg. Med. Chem. Lett. 2010;20:5827- 5830.
41. Stanway SJ, Purohit A, Woo LW, Sufi S, Vigushin D, et al. Phase I study of STX 64 (667 Coumate) in breast cancer patients: the first study of a steroid sulfatase inhibitor. Clin. Cancer Res. 2006;12:1585.
42. Kempen I, Papapostolou D, Thierry N, Pochet L, Counerotte S, Masereel B, et al. 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. 2003;88:1111-1118.
43. Mohamed FZ, EL-Deen IM, El-behary MM, Akaber KT. 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. 2013;3(6):481-485.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle3.com/review-history/49084