A novel schiff base derivative for effective treatment of azoxymethane induced colon cancer

Article *in* International Journal of Pharmaceutical Sciences and Research · August 2014

DOI: 10.13040/IJPSR.0975-8232.5(8).3544-50

9 authors, including:

Ayşegül Doğan
National Cancer Institute (USA), Frederick, NIH
26 PUBLICATIONS **148** CITATIONS
SEE PROFILE

Selami Demirci
National Institutes of Health
27 PUBLICATIONS **132** CITATIONS
SEE PROFILE

Kazim Sahin
Firat University
190 PUBLICATIONS **3,857** CITATIONS
SEE PROFILE

Fikrettin Sahin
Yeditepe University
391 PUBLICATIONS **7,724** CITATIONS
SEE PROFILE

IJPSR (2014), Vol. 5, Issue 8 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 21 January, 2014; received in revised form, 21 July, 2014; accepted, 30 July, 2014; published 01 August, 2014

A NOVEL SCHIFF BASE DERIVATIVE FOR EFFECTIVE TREATMENT OF AZOXYMETHANE INDUCED COLON CANCER

Aysegul Dogan [1], Nese Basak [2], Selami Demirci [1], Dilek Telci [1], Bülent Dede [3], Mehmet Tuzcu [4], Ibrahim Halil Ozercan [5], Kazım Sahin [6] and Fikrettin Sahin[1*]

Department of Genetics and Bioengineering, Faculty of Engineering and Architecture [1], Yeditepe University, Istanbul, Turkey
Department of Pharmaceutical Toxicology, University of Inonu [2], Malatya, Turkey
Süleyman Demirel University, Faculty of Sciences and Arts [3], Department of Chemistry, Isparta, Turkey
Department of Biology, Faculty of Science [4], Department of Pathology, Faculty of Medicine [5], Department of Animal Nutrition, Faculty of Veterinary Medicine [6] Firat University, Elazig Turkey.

Keywords:

Schiff base, colon cancer, azoxymethane, aberrant crypt foci

Correspondence to Author:

Fikrettin Sahin

Department of Genetics and Bioengineering, Faculty of Engineering and Architecture Yeditepe University Kayisdagi, Istanbul, Turkey

E-mail: fsahin@yeditepe.edu.tr

ABSTRACT: The field of cancer research has been emerged in recent years for the development of specific drugs to cancer treatment. New agents with the ability to provide efficient treatment by reducing side effects has led to new opportunities for improving agents for cytotoxic therapies. While there are several drugs for colon cancer treatment, researchers are trying to evaluate new agents or combinations of existing ones which can be used efficiently. Schiff bases with a wide range of variety and biological properties including anticancer activity might be used for colon cancer treatment. In the current study, a novel schiff base derivative synthesized by our group was tested in vivo for colon cancer. In a model of azoxymethane (AOM) induced colorectal cancer, chemopreventive properties of schiff base was also analyzed in rats. While AOM induced de novo crypt formation, adenocarcinoma and dysplasia development, schiff base application reduced the number of aberrant crypt foci (ACF), dysplasia or adenocarcinoma. Analysis of the intestinal mucosa showed that peritoneal administration of SB complex not only decreased the protein expression of COX-2, Bcl-2 and NF-κB but also enhanced the Bax expression suggesting the apoptotic and anti-proliferative effects for this compound. Our findings showed that SB complex might be used for the colorectal cancer treatment. Further studies are highly warranted to obtain additional insights and identify mode of action for the schiff base.

INTRODUCTION: After several years of efforts, the investigation of novel chemotherapeutic agents for cancer treatment has becoming a flourishing area against the traditional chemotherapy, which is well known for its serious side effects and toxicity. Cancer as a major health problem all around the world causes 1 in 4 deaths in the United States.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(8).3544-50

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(8).3544-50

Having considered data from statistical studies and published case studies in recent years, colorectal cancer has a high estimated death with 9% [1]. Colorectal carcinoma is the third most common type of cancer all over the world [2]. As an aggressive type of cancer; colorectal carcinoma is highly associated with morbidity [3].

Due to poor prognosis of the metastatic disease, survival time is reduced to 18 to 21 months [4]. Approximately 20% of patients diagnosed with the colorectal cancer has metastatic disease at the later stages [2]. Several chemotherapeutic agents based on fluorouracil have been used as the traditional therapy for colorectal cancer. Fluorouracil,

leucovorin and irinotecan or oxaliplatin combined therapy with bevacizumab are the first line treatment options for the colorectal carcinoma [5]. Patients with metastatic colorectal cancer have an estimated 8 month-survival period without chemotherapy. However fluorouracil treatment has increased the survival time upto 12 months. Irinotecan, oxaliplatin and bevacizumab have increased the survival time upto 21 months. Although the clinical efficacy of chemotherapeutics are being improved, the current treatment strategies for advanced colorectal cancer are only limited to the palliative therapy [6].

In recent years monoclonal antibodies have been effectively used for the combination therapy strategies developed for metastatic colorectal cancer [4]. New and alternative chemotherapy could be considered as a solution for the treatment of this cancer, resulting in the destruction of malignant cells, while having no non-specific toxicity on the other healthy cell types.

First characterized by Hugo Schiff in 1864, Schiff bases and their metal complexes are derived from the condensation reaction of primary amine and carbonyl compounds. Schiff bases were reported not only to have antibacterial and anti-fungal properties [7] but also to be effective against cancer cells [8]. Although there are different hypothesis for their anti-tumor activities, the exact mechanism of their action is not known as the anticancer effect can vary depending on the cancer type and the compound itself. It was suggested that the hydrogen bond formation between N–H and the nitrogen atoms of DNA and/or copper binding to the DNA or to the protein could provide the anti–tumor effect of Schiff bases [9-10].

In the current study a novel schiff base derivative: hetero-dinuclear copper (II) Mn(II) schiff base (SB) complex was tested on *in vivo* colorectal cancer model. We showed, for the first time, that SB complex decreased cancer progression when azoxymethane (AOM) induced colorectal cancer model were used in the *in vivo* studies suggesting an apoptotic and anti-proliferative activity for SB complex in colorectal carcinoma.

MATERIALS AND METHOD:

Preparation of Agents

Hetero-dinuclear copper(II) Mn (II) complex (SB) was synthesized and characterized by our group as described previously [11]. Prepared reagent was kept at room temperature in a light-protected tube until use.

Animals

Male Wistar rats (n= 84, 8 wk-old), weighing 200–215 g, were obtained from Firat University Research Center (Elazig, Turkey). Rats were kept in an environmentally controlled room at constant temperature (21 ± 1 ^{o}C) and humidity (50 ± 5%), under a 12h long light/dark period. The animals had free access to water and standard rodent diet ad libitum. The experiment was conducted under the protocol approved by the ethical committee of Firat University. All procedures were conducted in strict compliance with relevant laws, the Animal Welfare Act, Public Health Services Policy, and guidelines established by the Institutional Animal Care and Use Committee of the university.

In vivo Experimental Protocol

Rats were randomly divided into six groups, each consisting of 14 rats, as indicated in **Table 1**.

TABLE 1: *IN -VIVO* EXPERIMENTAL DESING.

Groups	Treatment	Period
Control	Ad libitum with rat standart diet	20 weeks
SB1 (1mg/kg)	Standart diet + peritoneal injection of SB complex	20 weeks
SB2 (2mg/kg)	Standart diet + peritoneal injection of SB complex	20 weeks
AOM (15mg/kg)	Standart diet + peritoneal injection of AOM only	20 weeks
AOM (15mg/kg)+S1	Standart diet + peritoneal injection of AOM +SB1	20 weeks
AOM (15mg/kg)+S2	Standart diet + peritoneal injection of AOM +SB2	20 weeks

Notes. SB1: Administration of SB complex at 1mg/kg of body weight, SB2: Administration of SB complex at 2mg/kg of body weight, AOM: Azoxymethane (15mg/kg).

The animals were injected subcutaneously with azoxymethane (Sigma Aldrich, AOM), a specific carcinogen that was diluted in saline, once per week (15 mg/kg body weight), over a 3-week period to induce colonic tumors. SB complex was given intraperitoneally as a candidate chemotherapeutic agent in two concentrations (1mg/kg and 2mg/kg of body weight) to the animals in every four days. Rats were decapitated twenty weeks after the starting of the experiment, and colorectal tissues were collected for histopathological and western blot analyses. Colons were opened flat overnight on hibond C paper for Aberrant Crypt Foci (ACF) analysis. A distal segment of normal-appearing colon (2 cm) free of neoplasms was dissected for western blot analysis (6/group).

Tumor histopathology

The colorectal tissues from each animal were collected and fixed in 20% neutral buffered formalin solution. The samples were then progressively dehydrated, inserted in paraffin, cut into small sections, and stained with hematoxylin and eosin for histological examination.

Western Blot Analysis

The protein levels in colon samples were determined by Western blotting as previously described [12]. Aliquots of colon tissues from 6 rats per group were pooled and homogenized in ice-cold lysis buffer (50 mM Tris, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, 5 mM EDTA, 2 mM PMSF and protease inhibitors) and then centrifuged at 15000 rpm for 60 minutes.

The concentration of protein in supernatants was determined using the Bio-Rad protein assay. Equal amounts of proteins (20 µg) were separated on SDS-PAGE gels, proteins were transferred to a nitrocellulose membrane using semi-dry transfer and the membrane was blocked with 5% skim milk, probed with Bcl-2, Bax, COX-2 and NF-κB antibodies (Abcam) and stained with secondary antibody (goat anti-mouse IgG). Western blot was repeated four times for each sample. Each membrane was re-probed with anti-β-actin antibody (Sigma). The band intensity was quantified by densitometric analysis using the image analysis system Image J (NIH, Bethesda, USA), and normalized to the β-actin band intensity.

Statistical Analysis

Data were statistically analyzed using one-way analysis of variance. The values of $P \leq 0.05$ were considered statistically significant. The continuous variables (moleculer biology) were analyzed by ANOVA using the PROC GLM procedure, whereas discrete variables (aberrant crypt foci (ACF), dysplasia and adenocarcinoma) were analyzed by chi-square test using the PROC FREQ procedure (SAS). Differences among the groups were attained by Duncan's multiple comparison. Statistical significance was declared at $P < 0.05$.

RESULTS:

Histopathological Results

Formation of precancerous ACF lesions was defined in order to assess the effect of Schiff base administration. No ACF developed in control group and 1mg/kg or 2mg/kg schiff base injected groups (SB1 and SB2). On the other hand AOM application caused the development of 160 crypts in the animals injected with 15mg/kg AOM for 3 weeks. However Schiff base administration decreased the ACF formation in a dose dependent manner. A similar correlation was found on the development of adenocarcinoma and dysplasia. No adenocarcinoma and dysplasia development was observed in control group and 1mg/kg or 2mg/kg schiff base injected groups (SB1 and SB2). AOM administration induced adenocarcinoma and dysplasia as expected. Schiff base application was significantly reduced the development of adenocarcinoma and dysplasia in a dose dependent manner **(Table 2)**.

Microscopically examination of the hematoxylin and eosin (H&E)-stained tissue sections demonstrated normal histological phenotype for control; SB1 and SB2 groups **(Figure 1 A-B-C)**. AOM application increased cell proliferation and destroyed the histological structure **(Figure 1 D)**. Schiff base administration overcame the negative effects of AOM by reducing the cell proliferation and tumor growth **(Figure 1 E-F)**.

We also assessed the effect of schiff base application on body weight change. Initial body weight (IBW) and final body weight (FBW) were measured and calculated. Body weight was not

significantly changed in either control or experimental groups (data not shown).

TABLE 2. THE EFFECTS OF SCHIFF BASE APPLICATION ON THE DEVELOPMENT OF ABERRANT CRYPT FOCI (ACF), DYSPLASIA, AND ADENOCARCINOMA IN THE INDICATED GROUP OF RATS

Groups	No. Of ACF	1 crypt	2 crypt	3 crypt	≥4 crypt	Displasie (%)	Adenocarsinoma (%)
Control	0,00	0,00	0,00	0,00	0,00	0,00	0,00
SB1	0,00	0,00	0,00	0,00	0,00	0,00	0,00
SB2	0,00	0,00	0,00	0,00	0,00	0,00	0,00
AOM	160,50	69,86	54,43	23,07	13,14	50,0	57,14
AOM+SB1	104,93	45,86	29,00	19,21	10,86	28,6	35,71
AOM+SB2	92,93	37,86	28,57	16,79	9,71	21,4	28,57

Notes. SB1: Administration of SB complex at 1mg/kg of body weight, SB2: Administration of SB complex at 2mg/kg of body weight, AOM: Azoxymethane (15mg/kg).

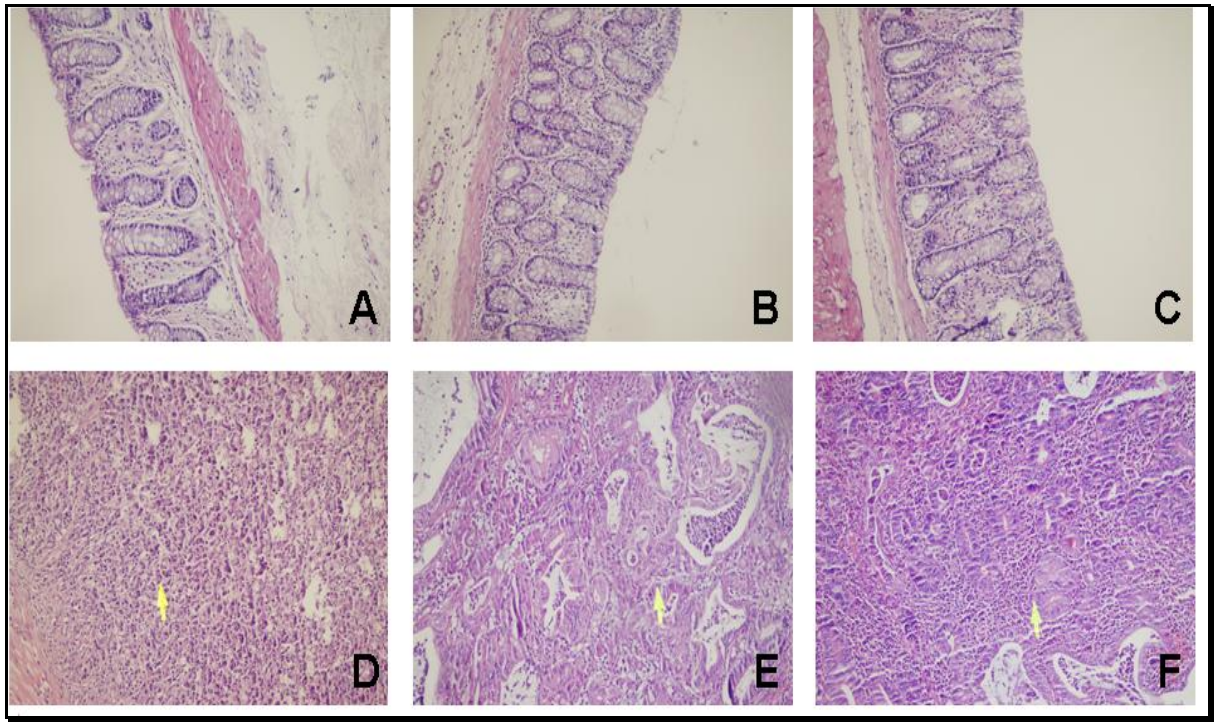

FIGURE 1. REPRESENTATIVE HISTOPATHOLOGY OF HEMATOXYLIN AND EOSIN STAINED COLORECTAL TISSUE SECTIONS (A) CONTROL GROUP, (B) SB1, (C) SB2, (D) AOM GROUP, (E) AOM + SB1 GROUP, (F) AOM + SB2 GROUP.
Notes. Magnification 400x

Western Blot Analysis

Bcl-2, Bax, NF-κB, Cox-2 protein expression levels were determined to find out the possible molecular mechanism for the prevention of AOM-induced colorectal carcinogenesis by Schiff base application. AOM administration caused a significant 2 fold increase in the anti-apoptotic Bcl-2 protein levels which was brought down by 58% by the administration of 1 mg/kg SB and 67% by the administration of 2 mg/kg SB ($p<0.05$) **(Figure 2A)**. In contrast, the administration of SB led to an average of 30% significant increase in the pro-apoptotic Bax protein levels **(Figure 2B)**. There was an approximate 1.8 fold increase in the inflammation markers NF-κB and Cox-2 protein levels in AOM administered animals ($p<0.05$).

Treatment with SB at both concentrations resulted in a significant 54% decrease in NF-κB expression **(Figure 2C)** while a significant 46% reduction was recorded for Cox-2 protein expression **(Figure 2D)**. Administration of SB at 1 and 2 mg/kg alone did not lead to any significant change in the protein

levels of Bcl-2, Bax, NF-κB and Cox-2 when compared to non-treated control group animals.

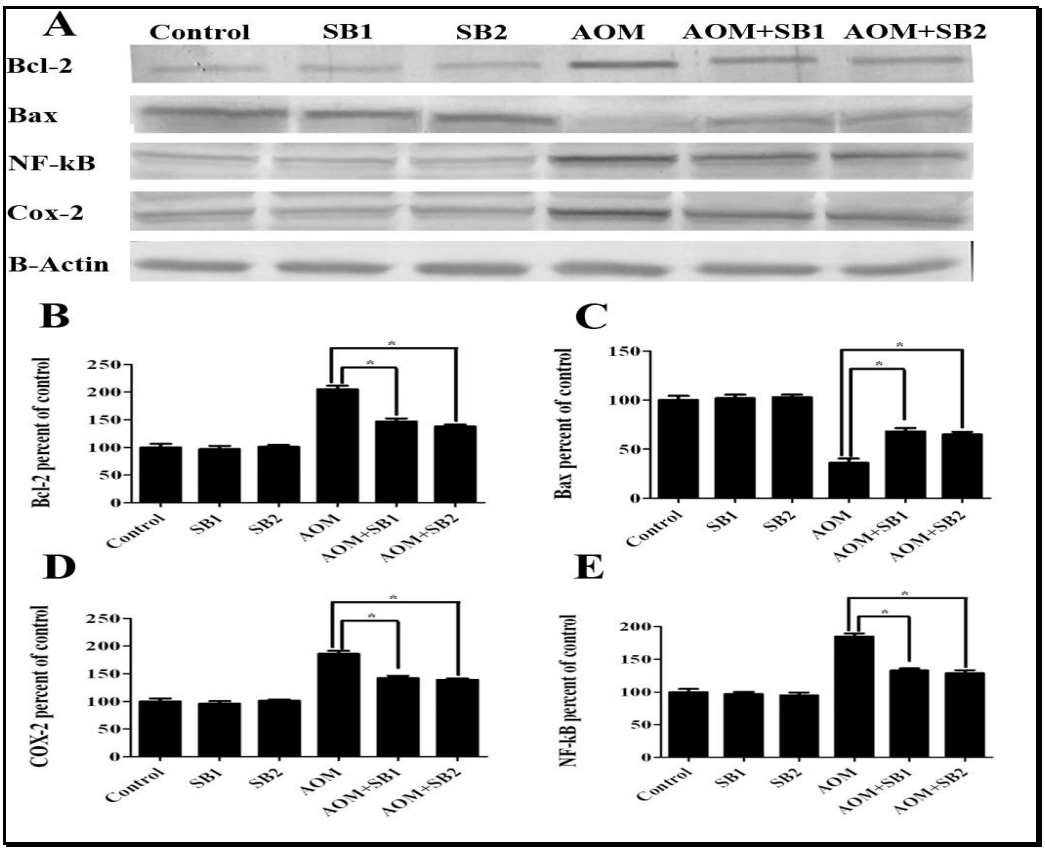

FIGURE 2. EFFECTS OF SCHIFF BASE ON PROTEIN EXPRESSION LEVELS. DATA ARE MEANS OF QUADRUPLETS OF ASSAYS AND EXPRESSED AS RELATIVE TO CONTROL (%). VALUES ARE MEANS ± STANDARD ERROR OF THE MEAN. ACTIN WAS INCLUDED TO ENSURE EQUAL PROTEIN LOADING. (*P<0.05 FISHER'S COMPARISON TEST) (A) GEL IMAGE OF WESTERN BLOT ANALYSIS AND NORMALIZED PROTEIN EXPRESSION LEVELS OF (B) Bcl-2, (C) Bax, (D) Cox-2, and (E) NF-κB.

Notes. SB: Schiff base SB1: 1mg/kg , SB2: 2mg/kg , AOM: Azoxymethane (15mg/kg)

DISCUSSIONS: Colon cancer is one of the most common cancers that develops in several stages characterized by hyperproliferation and crypt formation. Metastases to lymph nodes is the main cause for the morbidity [13]. Current practices for colon cancer treatments encompass combination therapies including fluoropyrimidines, irinotecan or oxaliplatin with side effects depending on the application regimen. Although there are several agents used for colon cancer treatment, new options are required to provide efficient cytotoxic therapy preventing metastasis and reducing mortality.

Investigation of new chemotherapeutics is of great interest in colon cancer research to provide efficient and safe therapy minimizing side effects. Schiff bases with their broad biological activity could be useful for cancer treatment. In the current study we tested the effects of a novel schiff base derivative on the AOM established colorectal cancer model. ACF, adenocarcinoma and dysplasia development were significantly diminished by Schiff base administration. The results from animal experiments highlight that effect of Schiff base differ based on applied concentration. ACFs are preneoplastic lesions for colorectal cancer and more than four aberrant crypt formations can be accepted as colon carcinogenesis [14].

Schiff base application reduced the number of ACFs. Proliferating cells and tumor growth rate were also reduced in the Schiff base group compared to control. The results confirm that Schiff base can inhibit the chemically induced colon carcinogenesis in the rat animal models. Cyclooxygenase-2 (COX-2) as an enzyme

responsible for prostaglandin biosynthesis is expressed high levels in cancer situations [15]. Drugs targeting COX-2 those related with NSAIDs (nonsteroidal anti-inflammatory drugs) were found to reduce colorectal cancer development [16]. In the study COX-2 protein expression level was analyzed to observe whether Schiff base application has a protective effect against colon cancer. Although Schiff base application significantly decreased the COX-2 expression, further studies are required to elucidate the exact mechanism for COX-2 inhibition.

In order to detail how COX-2 inhibition is regulated we tested the nuclear factor-kappa B (NF-κB) expression which mediates the COX-2 expression induced by inflammation [17]. The results from western blot studies indicates that a similar correlation exist about expression patterns of COX-2 and NF-κB. NF-κB was also reduced in treatment groups. NF-κB as a transcription factor is involved in many responses including inflammation, cell proliferation and apoptosis [18]. The similarity of western blot results might be explained by these interactions. In addition, inhibition of cell proliferation in the treatment groups is highly associated with apoptosis. A strong correlation was found between anti-apoptotic Bcl-2 and apoptotic Bax genes. While Bax expression was enhanced, Bcl-2 expression was decreased as expected indicating the induction of apoptosis by Schiff bases.

CONCLUSIONS: Taken together, results of the current study clearly demonstrated that Schiff base attenuates AOM induced carcinogenesis by regulating the inflammatory and apoptotic pathways. This is the first study showing the effect of a novel Schiff base hetero-dinuclear copper (II) Mn (II) complex on colon carcinogenesis. This study will bring the usage of this Schiff base a step closer to the clinical applications as a chemotherapeutic agent. Despite successful results of the study, several researches about action of mechanism should be completed to increase efficiency of the drug.

ACKNOWLEDGEMENTS: This work was partially supported by grants from Turkish Academy of Science, Ankara (TUBA) (K.S.).

REFERENCES:

1. Siegel R, Naishadham D and Jemal A: Cancer statistics, 2013. CA Cancer J Clin 2013; 63: 11-30.
2. Bray F, Ren JS, Masuyer E and Ferlay J: Global estimates of cancer prevalence for 27 sites in the adult population in 2008. Int J Cancer 2013; 132: 1133-1145.
3. Bardelli A and Siena S: Molecular mechanisms of resistance to cetuximab and panitumumab in colorectal cancer. J Clin Oncol 2010; 28: 1254-1261.
4. Poston GJ, Figueras J, Giuliante F, Nuzzo G, Sobrero AF, Gigot J-F, Nordlinger B, Adam R, Gruenberger T and Choti MA: Urgent need for a new staging system in advanced colorectal cancer. J Clin Oncol 2008; 26: 4828-4833.
5. Van Cutsem E, Köhne C-H, Hitre E, Zaluski J, Chang Chien C-R, Makhson A, D'Haens G, Pintér T, Lim R and Bodoky G: Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. N Engl J Med 2009; 360: 1408-1417.
6. Schrag D: The price tag on progress-chemotherapy for colorectal cancer. N Engl J Med 2004; 351: 317-319.
7. Nair MS, Arish D and Joseyphus RS: Synthesis, characterization, antifungal, antibacterial and DNA cleavage studies of some heterocyclic Schiff base metal complexes. J Saudi Chem Soc 2012; 16: 83-88.
8. Arulmurugan S, Kavitha HP and Venkatraman B: Biological activities of schiff base and its complexes: a review. J Chem 2010; 3: 385-410.
9. Chaviara AT, Christidis PC, Papageorgiou A, Chrysogelou E, Hadjipavlou-Litina D and Bolos C: In vivo anticancer, anti-inflammatory, and toxicity studies of mixed-ligand Cu (II) complexes of dien and its Schiff dibases with heterocyclic aldehydes and 2-amino-2-thiazoline. Crystal structure of [Cu (dien)(Br)(2a-2tzn)](Br)(H_2O). J Inorg Biochem 2005; 99: 2102-2109.
10. Qiao X, Ma Z-Y, Xie C-Z, Xue F, Zhang Y-W, Xu J-Y, Qiang Z-Y, Lou J-S, Chen G-J and Yan S-P: Study on potential antitumor mechanism of a novel Schiff Base copper (II) complex: synthesis, crystal structure, DNA binding, cytotoxicity and apoptosis induction activity. J Inorg Biochem 2011; 105: 728-737.
11. Dede B, Karipcin F and Cengiz M: Novel homo-and hetero-nuclear copper (II) complexes of tetradentate Schiff bases: synthesis, characterization, solvent-extraction and catalase-like activity studies. J Hazard Mater 2009; 163: 1148-1156.
12. Dogukan A, Tuzcu M, Agca CA, Gencoglu H, Sahin N, Onderci M, Ozercan IH, Ilhan N, Kucuk O and Sahin K: A tomato lycopene complex protects the kidney from cisplatin-induced injury via affecting oxidative stress as well as Bax, Bcl-2, and HSPs expression. Nutr Cancer 2011; 63: 427-434.
13. Fan X-J, Wan X-B, Yang Z-L, Fu X-H, Huang Y, Chen D-K, Song S-X, Liu Q, Xiao H-Y and Wang L: Snail promotes lymph node metastasis and Twist enhances tumor deposit formation through epithelial-mesenchymal transition in colorectal cancer. Hum Pathol 2013; 44: 173-180.
14. Rao CV, Newmark HL and Reddy BS: Chemopreventive effect of squalene on colon cancer. Carcinogenesis 1998; 19: 287-290.
15. Kraus S, Naumov I and Arber N: COX-2 active agents in the chemoprevention of colorectal cancer. Recent Results Cancer Res 2013; 191: 95-103.
16. Tinsley HN, Grizzle WE, Abadi A, Keeton A, Zhu B, Xi Y and Piazza GA: New NSAID targets and derivatives for

colorectal cancer chemoprevention. Recent Results Cancer Res 2013; 191: 105-120.

17. Plummer SM, Holloway KA, Manson MM, Munks RJ, Kaptein A, Farrow S and Howells L: Inhibition of cyclo-oxygenase 2 expression in colon cells by the chemopreventive agent curcumin involves inhibition of NF-kB activation via the NIK/IKK signalling complex. Oncogene 1999; 18: 6013-6020.
18. Zubair A and Frieri M: Role of nuclear factor-κB in breast and colorectal cancer. Curr Allergy Asthma Rep 2013; 13: 44-49.

How to cite this article:
Dogan A, Basak N, Demirci S, Telci D, Dede B, Tuzcu M, Ozercan IH, Sahin K and Sahin F: A Novel Schiff Base Derivative for Effective Treatment of Azoxymethane Induced Colon Cancer . Int J Pharm Sci Res 2014; 5(8): 3544-50.doi: 10.13040/IJPSR.0975-8232.5 (8).3544-50.