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Antioxidant and Cytotoxic Activity Studies in Series of Higher Amino Acid Schiff Bases

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Highlights

- DPPH scavenging ability of the higher amino acid Schiff bases was investigated.
- *In vitro* cytotoxicity of these Schiff bases was tested against cancer and human normal cells.
- Schiff base 2b killed 90 percent of MCF-7 cells at concentration of 100 µM.

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Abstract

In this work, the antioxidant activity of the higher amino acid Schiff bases, which were prepared as the monosodium salts (**1a**–**3a**) and the neutral forms (**1b**–**3b**) was determined by DPPH scavenging assay. In pure MeOH solution, the scavenging ability of Schiff bases **1a-3a** were higher than **1b-3b**, but lower than ascorbic acid. The activity followed the order 3 (a,b) > 2 (a,b) > 1 (a,b). On the other hand, Schiff bases **2a** and **3a** behaved as the most effective scavengers of the DPPH radical in methanol-water mixture (v:v, 1:3). And, they were found to be have lower SC50 values in this mixture compared to pure methanol. In vitro cytotoxicity of these Schiff bases was studied against human cervical cancer cells (HeLa), human breast adenocarcinoma cells (MCF-7), and human normal embryonic kidney cells (HEK293). For HeLa cell line, Schiff bases **1a-3a** exhibited a litttle high activity than **1b**, but very low activity than doxorubicin. Schiff bases **2b** and **3b** had no cytotoxicity against HeLa cell. For MCF-7 cell line, Schiff bases **1a**, **3a**, **1b** and **3b** nearly were inactive at 100 µM, whereas **2a** increased cell proliferation in the all tested concentration range. Differently, Schiff base **2b** showed the highest cytotoxicity and killed 90 percent of MCF-7 cells at concentration of 100 µM. For HEK-293, doxorubicin was strongly cytotoxic. Despite this, Schiff bases **1a**, **3a** and **3b** were inactive, whereas the others showed little weak toxicity.

1. INTRODUCTION

Endogenous reactive oxygen species (ROS: $O^{\cdot}$,$OH^{\cdot}$, $O_2^{\cdot-}$ and H_2O_2) are naturally formed in the life of aerobic organism. Exogenous species occur as a result of toxic agents, drugs or different lifestyle choices like as smoking and burnt food, etc. [1]. The excess ROS production becomes toxic, and this damages structure and function of biological molecules like as lipids, carbohydrates, proteins, DNA and nucleic acids in body metabolism, and causes degenerative diseases such as aging, diabetes, inflammatory, cardiovascular, autoimmune and cancer [2-4]. Also, neurodegenerative diseases as Alzheimer's and Parkinson's have been found to be related to an increase in quantity of ROS inside the human body [5]. Antioxidants react with these free radicals, terminate their chain reactions and minimize their harmful effects in metabolism [6]. They also protect the quality of food stuffs [7]. Natural antioxidants isolated from aromatic plants are flavonoids, tannins, phenolic acids, alkaloids, chlorophyll derivatives, carotenoids and tocopherols. There are also some synthetic phenolic antioxidants as butylated hydroxy-toluene, butylated hydroxy-anisole and tert-butylhydroquinone [8].

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Cancer is a group of diseases which is characterized by uncontrolled cell proliferation and disruption of vital tissues [9]. The mutation in DNA is the main reason for all common cancer types. Otherwise, oxidative stress due to the excess production of ROS has a significant role in carcinogenesis [10]. In the treatment of cancer, different methods such as chemotherapy, radiotherapy, immunotherapy etc. are utilized. Recently, antioxidants have been reported to induce apoptosis process in the cancer cells by decreasing the cellular ROS levels [11]. Among them chemotherapy is one of the most common method, and antineoplastic drugs are used in the chemotherapy of tumors. Metal-based drugs cisplatin and its analogues (nedaplatin, lobaplatin and heptaplatin) are highly effective drugs, which are succesfully utilized for the treatment of solid tumors as well as testicles, ovarian, melanoma and breast [12-13]. Doxorubicin is also one of anti-cancer antibiotics. It is generally used for lung and esophageal carcinoma, osteosarcoma, Hodgkin and Non-Hodgkin lymphoma [14]. But, the usage of all these chemotherapeutics are often limited by several types of side effects such as dose-limiting toxicity, nephrotoxicity, neurotoxicity, ototoxicity, and cardiotoxicity [15-16]. The other important problem is the occurrence of resistance to drugs in tumor cells during chemotherapy [17]. For these reasons, there is a need for new drugs with higher activity and less toxicity than present antineoplastic drugs, and thus the design of new agents has become the main aim of research in medicinal chemistry.

Amino acid Schiff bases obtained from salicylaldehyde and α-amino acids, as glycine, alanine, phenylalanine, valine, histidine, and etc. have been extensively synthesized because of being model compounds of a coenzyme of vitamin B6 [18-22]. These Schiff bases are one of the class of compounds possessing a broad spectrum of pharmacological activities such as antibacterial [23], antifungal [24], anti-inflammatory [25], enzyme inhibition [26] and antioxidant activity [27]. Their metal complexes containing platinum [28], ruthenium [29], palladium [30], nickel [31] and lanthanum [32] have shown to be promising good antitumor, serum albumin and DNA-binding activities. In recent years, researchers have reported a novel series of the Schiff bases derived from alkanoic acid molecules [$H_2N{-}(CH_2)_n{-}COOH$, where $n \geq 3$] [33-35]. But, information on their activities as antioxidant and antitumor is still rare.

2. MATERIAL METHOD

2.1. Materials

2,2-diphenyl-1-picrylhydrazyl (DPPH) (Aldrich), L-ascorbic acid (Carlo Erba), Dulbecco's Modified Eagle's Medium (DMEM) (Gibco), fetal bovine serum (FBS) (Gibco), penisilin-streptomisin (PAA), doksorubicin (Tocris) were used without purification. Absolute methanol and ethanol were purchased from Sigma. Two different human carcinoma cell lines (HeLa, human cervical cancer and MCF-7, human breast adenocancer) were obtained from Molecular Biology and Genetic Department of Bilkent University and Biochemistry Department of Middle East Technical University, respectively. Normal human cell line (HEK293, human embryonic kidney cell) was obtained from Biotechnology Institute of Ankara University.

2.2. Synthesis of the Higher Amino Acid Schiff Bases

The monosodium-Schiff bases **(1a–3a)** and the neutral-Schiff bases **(1b–3b)** were synthesized and characterized in our previous work [36].

2.3. DPPH Radical Scavenging Activity

The activity was evaluated by following the reported method [37]:
Sample solutions (2 mg/mL) of the monosodium-Schiff bases were prepared separately in doubly distilled water and methanol. Under same condition, 2 mg of the neutral-Schiff bases was dissolved in 1 mL of methanol. And, a solution of DPPH (0.1 mM) was prepared in methanol. A volume of 1 mL of DPPH was added to different volumes of sample solutions of compounds (2 mg/mL), and it made up to a final volume of 4 mL using solvent. The tubes was shaken vigorously and kept in dark at room temperature. After 30 min, the absorbance of the samples was recorded at 517 nm by using Analytik Jena Specord 200 spectrophotometer in steps of 1 nm using a 1-cm-thick quartz cell. Methanol was used for the baseline

correction. DPPH solution and ascorbic acid were used as control and standard. All determinations were performed in triplicate.

DPPH scavenging activity was calculated by using Equation (1):

$$\%DPPH\ scavenging = {}^{(A0 - A1)}/_{A0} \times 100 \quad (1)$$

where A_0 and A_1 are the absorbance of control and sample solution. % scavenging was plotted against the different concentrations used, and SC_{50} value was calculated from the graph. SC_{50} (mg sample per mL) is the concentration of the samples that causes 50% scavenging of DPPH radical.

2.4. Cell Culture

Three different human cell lines were cultured in DMEM supplemented with 10% FBS, 1% penicillin-streptomycin. Cells were maintained at 37 ºC in a humidified atmosphere of 5% CO_2 (Sanyo) in air. The culture medium was changed every 2 days until the cell culture reached 70–80% confluence. When cells reached 70–80% confluence, they were treated with Schiff bases and doxorubicin at varying concentrations. At the end of two days, three different areas were chosen randomly, and an image of the cells was taken using phase-contrast microscope (Leica). Blebbing and normal cells were counted in the photographs by microscope camera (Leica DFC 420 C). The percentage of cells with blebs was calculated by dividing the number of cells with blebs by the total number of cells.

2.5. Real Time Monitoring of Cytotoxicity

An xCELLigence Real-Time Cell Analyzer (RTCA) DP system (Roche) was used to monitor the viability and migration of cells (HeLa, MCF-7 and HEK293), as previously described [38]. Briefly, $8 \times 10^3 – 1 \times 10^4$ cells were plated per well of an E-plate. Cell growth and/or viability was monitored every 15 min for 20 h. After 20 h, the existing medium was replaced with fresh medium (DMEM + 10% FBS + 1% penisilin-streptomisin). When Cell Index was in the range of 1–1.5, the cells were incubated with Schiff bases and doxorubicin at varying concentrations. As an indicator of cell detachment, the cell index was recorded continuously for 48 h and analyzed by RTCA DP system. HeLa, MCF-7 and HEK293 cells were monitored for another 48 h, as a control.

2.6. Statistical Analysis

All values were expressed as the mean ± s.e.m and analyzed by Student's t test, and ANOVA. $P < 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. DPPH Assay

To evaluate the antioxidant activity of Schiff bases (Figure 1), they reacted with a stable $DPPH^{\cdot}$ radical in pure methanol and methanol-water (v:v, 1:3) solutions. The reaction was monitored by the decrease in absorbance of DPPH at 517 nm. The percentages of DPPH radical scavenging were tabulated in Tables 1 and 2.

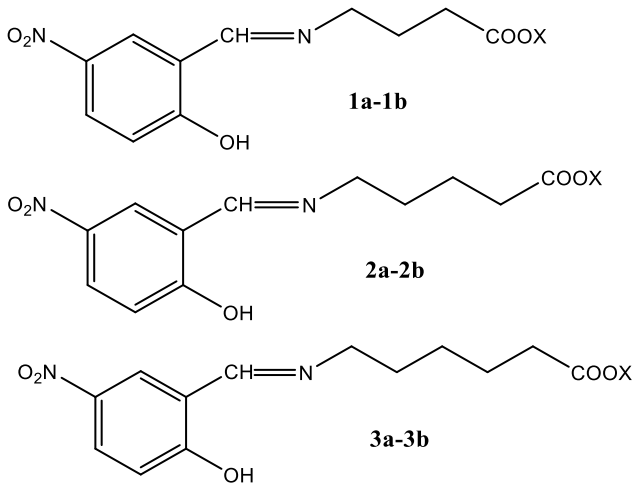

Figure 1. *The structures of the higher amino acid Schiff bases*

As can be seen in Table 1, when the concentration of the compounds was increased, the scavenging percentage was also increased, which points out the lower SC_{50} value. Schiff bases (**1a–3a**) showed good antioxidant activities of SC_{50} values (5.04, 3.79 and 3.34 μM) in MeOH solution, comparable with that of **1b–3b** (9.05, 7.52 and 4.82 μM). This indicated that the scavenging potency of the neutral-Schiff bases is lower than the monosodium-Schiff bases, despite the activity is only dependent on the presence of hydroxyl group. It may be explained by an electron-withdrawing effect of carboxyl group in **1b–3b** [39]. The scavenging activity for Schiff bases decreased in the order: **3a** >**2a** >**1a** and **3b** >**2b** >**1b**. This may be due to the increasement of a number of an electron donating (CH_2) group in structure, which increase the resonance ability and radical scavenging potency of phenoxyl radical. This result may be associated to the lipophilic character of alkyl chain. Porter suggested that lipophilic antioxidants show more antioxidant activity in polar mediums [40]. Besides, it has been reported that monophenols hindered sterically by alkyl groups have a high activity [41]. Under the same test conditions, SC_{50} value of ascorbic acid was found to be < 0.14 μM. Different SC_{50} values were presented in literature for ascorbic acid in MeOH solution such as 11.8 μM [42] and 46.81 μM [43], which were related to used DPPH concentration. SC_{50} values of six Schiff bases were higher than ascorbic acid corresponding to their medium antiradical efficiency. It may be resulted from strong electron-withdrawing effect of nitro group in Schiff bases. Because nitro group can cause the poor resonance in phenoxyl radical and decrease the scavenging potency [44].

Table 1. *DPPH scavenging activity of all the Schiff bases in MeOH*

Compound	% Scavenging average[a]							SC_{50} mg/mL (μM)
	Concentration (mg/mL)[b]							
	0.10[b]	0.20	0.40	0.60	0.80	1.20	1.60	
1a	30.05 [a] ±0.28	32.69 ±0.31	36.12 ±0.07	39.10 ±0.32	41.56 ±0.44	44.53 ±0.50	54.96 ±0.52	1.38 (5.04)
2a	28.78 ±0.84	31.60 ±0.72	36.71 ±0.79	40.45 ±0.72	46.22 ±0.32	51.43 ±0.33	59.61 ±0.62	1.09 (3.79)
3a	29.29 ±0.35	31.92 ±0.11	38.73 ±0.22	42.73 ±0.26	46.42 ±0.41	53.64 ±0.21	61.47 ±0.18	1.01 (3.34)
1b	24.46 ±0.61	26.02 ±0.02	28.34 ±0.61	30.35 ±0.23	32.42 ±0.22	37.40 ±0.14	42.24 ±0.76	2.28 (9.05)
2b	25.72 ±0.25	27.49 ±0.06	29.70 ±0.25	32.22 ±0.12	35.29 ±0.31	39.17 ±0.11	45.29 ±0.57	2.00 (7.52)
3b	26.55 ±0.24	30.92 ±0.46	33.92 ±0.77	37.86 ±0.19	41.09 ±0.08	47.29 ±0.57	53.71 ±0.50	1.35 (4.82)
Ascorbic acid	0.025[b]	0.050	0.10	0.15	0.20	0.30	0.40	
	59.52 [a] ±0.18	60.53 ±0.08	61.27 ±0.33	63.01 ±0.01	63.91 ±0.25	66.57 ±0.53	69.09 ±0.24	< 0.025 (< 0.14)

In an aqueous methanolic solution, SC_{50} values of the monosodium-Schiff bases (**1a–3a**) and ascorbic acid were determined as 2.85 µM, 2.15 µM, 1.85 µM and 2.61 µM, respectively. The scavenging activity followed the order: **3a** >**2a** > ascorbic acid >**1a**. From Tables 1 and 2, it was clear that Schiff bases (**1a–3a**) have lower SC_{50} values in methanol-water mixture in comparison to pure methanol. It is well known that radical scavenging mechanism is influenced by the solvent polarity and its hydrogen bonding ability [8]. The strong intermolecular hydrogen bonding between methanol and water molecules could facilitate the interaction between Schiff bases and DPPH radical, and enhance the intermolecular hydrogen transfer from phenol moiety of compound to radical. The comparison of SC_{50} values of compounds in pure MeOH and aqueous methanolic solution was given in Figure 2.

Table 2. *DPPH scavenging activity of the monosodium-Schiff bases in MeOH-water mixture (v:v, 1:3)*

Compound	% Scavenging average[a]							SC_{50} mg/mL (µM)
	Concentration (mg/mL)[b]							
	0.10[b]	0.20	0.40	0.60	0.80	1.20	1.60	
1a	33.03 [a] ±0.02	36.49 ±0.54	42.52 ±0.56	46.99 ±0.08	52.08 ±0.12	59.92 ±0.52	66.87 ±0.10	0.78 (2.85)
2a	39.35 ±0.53	41.37 ±0.40	45.96 ±0.36	50.41 ±0.16	54.06 ±0.08	62.74 ±0.76	67.77 ±0.90	0.62 (2.15)
3a	40.25 ±0.39	42.50 ±0.04	46.52 ±0.14	51.98 ±0.17	55.50 ±0.26	63.64 ±0.23	68.21 ±0.39	0.56 (1.85)
Ascorbic acid	0.025[b]	0.050	0.10	0.15	0.20	0.30	0.40	
	39.89 [a] ±0.36	40.47 ±0.62	42.17 ±0.14	43.21 ±0.31	44.91 ±0.26	46.66 ±0.23	48.00 ±0.18	0.46 (2.61)

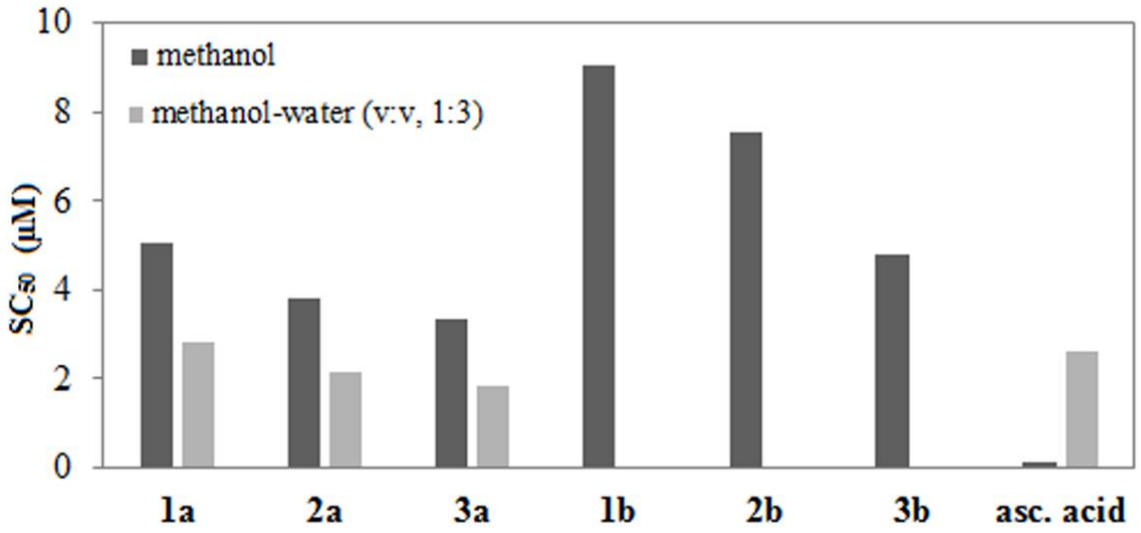

Figure 2. *Comparable chart for SC_{50} values (µM) of Schiff bases. The set concentration of DPPH was 25 µM. Data are the means of three independent experiments*

The obtained DPPH assay results indicated that Schiff bases **1a-3a** were found to be more active than **1b-3b**, but less active than ascorbic acid in pure MeOH solution. The activity increased with an increasement of alkyl chain in series, and it followed the order 3 (**a,b**) > 2 (**a,b**) > 1 (**a,b**). Besides, the scavenge ability of the monosodium-Schiff bases was in the order: **3a**>**2a**> asc. acid >**1a** in an aqueous methanolic solution.

Literature survey has showed that o-vanillylidene-L-histidine Schiff base and its copper(II) complex exhibited good scavenging ability in methanol with SC_{50} values of 0.6 mg/mL and 0.3 mg/mL [45]. Some sodium-Schiff bases derived from alanine, valine and phenylalanine were found to be have SC_{50} value of 1.38 mg/mL, 3.11 mg/mL and 0.45 mg/mL in methanol, respectively [18]. A novel Schiff base including s-allyl cystiene and methionine had lower SC_{50} value (52.5 µg/ mL), which was related to a higher radical

scavenging activity [46]. Oxo-vanadium complexes of N-salicyldene-amino acid sodium sulfonate ligands (amino acid = alanine, leucine, glycine and tryptophan) were also reported to have high scavenging potential in methanol [47]. Despite this, Schiff base derived from gabapentin and its Co(II), Ni(II), Cu(II) and Zn(II) complexes acted to inactive in ethanol [1]. From these results, it was seen that Schiff bases (**1a–3a**) and (**1b–3b**) will be efficient DPPH radical scavengers in literature.

3.2. Cytotoxicity Assay

In vitro cytotoxicities of Schiff bases were investigated against two human cancer cell lines (HeLa and MCF-7), and a normal human cell line (HEK-293). Cells treated with EtOH were used as a solvent control.

The morphological changes in HeLa cancer cell were confirmed by phase contrast microscopy (Figure 3). From the results in Figure 4, it was noted that the monosodium-Schiff bases **1a–3a** are less active cytotoxicity toward HeLa cell line with the cell-viability values between 80% and 87% in compared with doxorubicin. They showed dose-dependent antiproliferative effect within the concentration range of 100-10 μM (**1a**), 100 μM (**2a**) and 100-1 μM (**3a**). The neutral-Schiff base **1b** had low cytotoxicity toward HeLa cell line in the concentration range of 30-1 μM (98-88% viability), while Schiff base **2b** demonstrated inactivity (Figure 4a). Schiff base **3b** increased the cell proliferation at concentration of 30 μM and 100 nM, but it behaved inactive between 10 μM and 1 μM (Figure 4b).

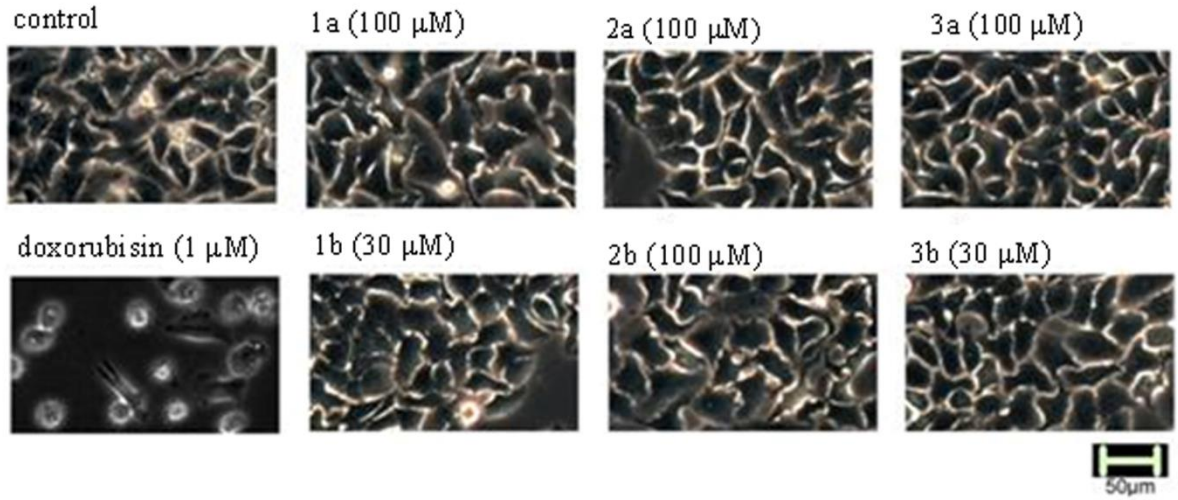

Figure 3. *Phase contrast photographs of HeLa cells at 48 hours (x200)*

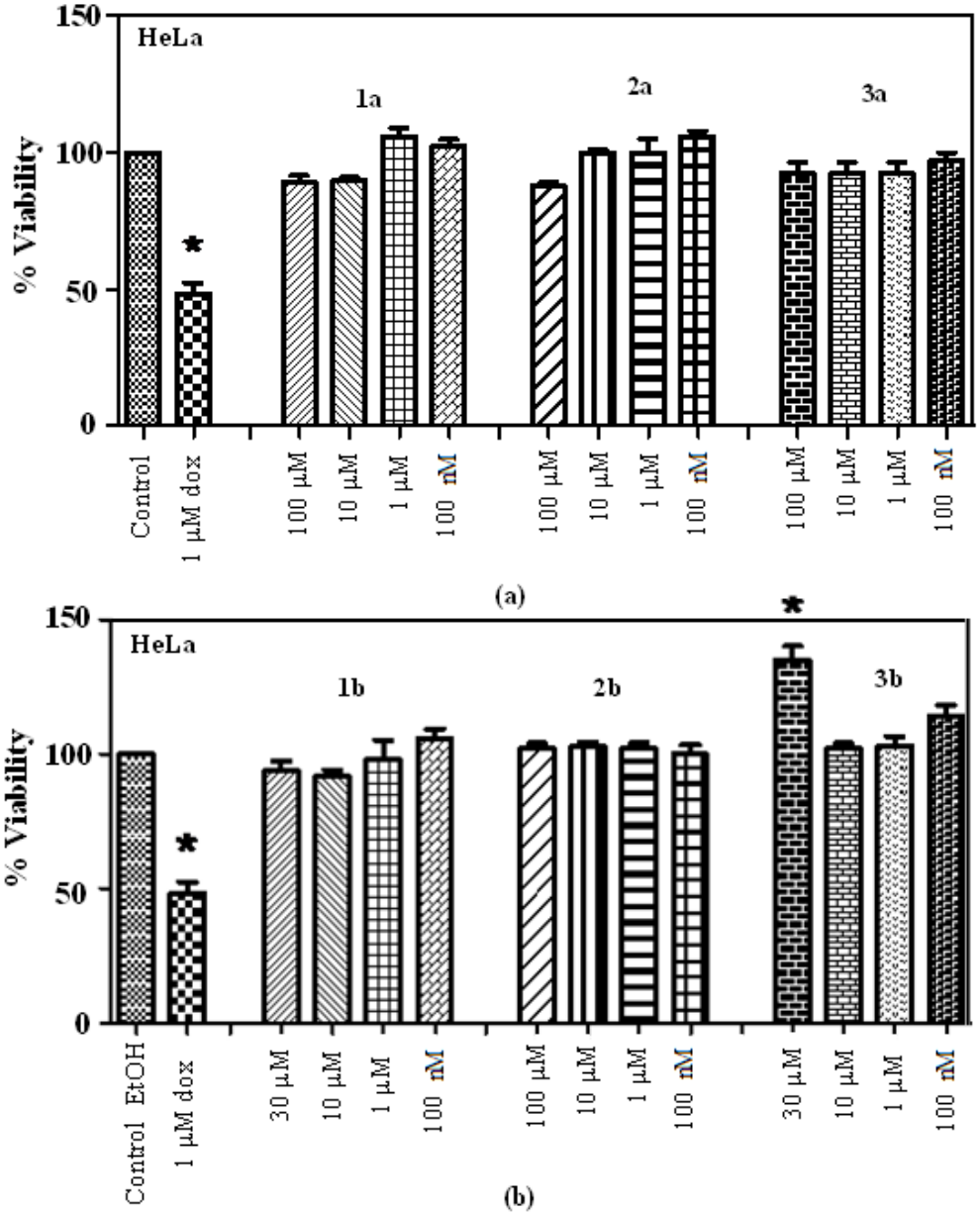

Figure 4. *The evaluation of cytotoxic activity of Schiff bases against HeLa carcinoma cell line; a) the monosodium-Schiff bases (**1a-3a**), b) the neutral-Schiff bases (**1b-3b**). Dox, is Doxorubicin. * shows significant difference from control group. (One-way analysis of ANOVA, Tukey, $p < 0.05$, $n = 3$)*

The changes in MCF-7 cell line, which were pretreated with Schiff bases and doxorubicin were given in Figure 5. The monosodium-Schiff bases **1a** and **3a** showed no cytotoxicity against MCF-7 carcinoma cell line (Figure 6a) at concentration of 100 μM, however the cell proliferation increased with a decrease in their concentration. Schiff base **2a** displayed the worst cytotoxicity against MCF-7. It caused a increase in the cell proliferation in the concentration range of 100 μM-100 nM. Neutral-Schiff base **1b** was inactive against MCF-7 between 30 μM and 10 μM, but the concentration ≤ 1 μM, it slightly increased the viability of cell line. Schiff base **3b** did not have any cytotoxicity within the concentration range of 30 μM-100 nM (Figure 6b). When tested against MCF-7 cell line, Schiff base **2b** exhibited dual effect. This means that it achieved to inhibit the growth of carcinoma cell at high concentration, and failed to cause antiproliferation on cell at low concentration. At concentration of 100 μM, it showed very significant activity with the cell-death value of 90%. But, it increased the cell proliferation at 1 μM compared with that of doxorubicin (1 μM, 18% viability).

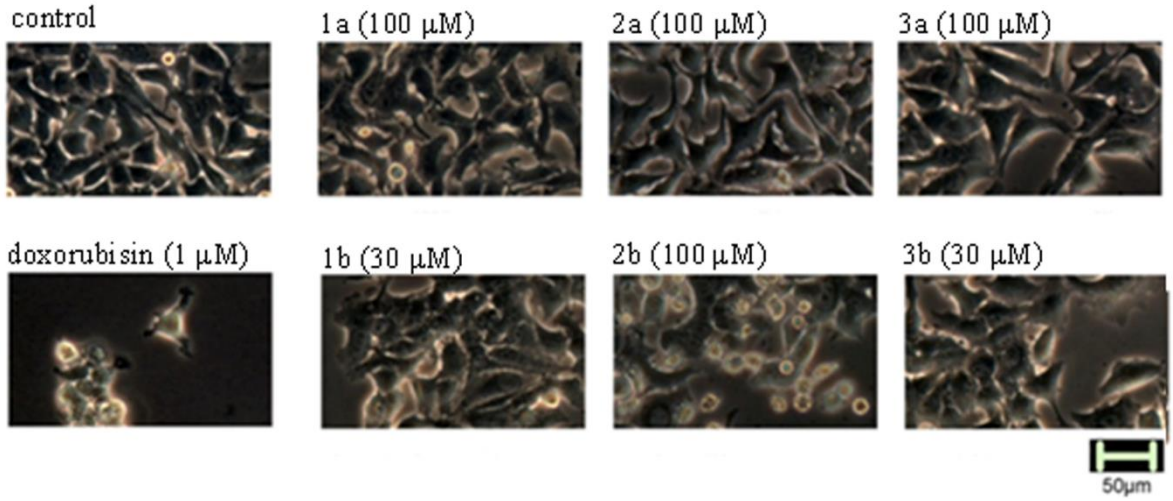

Figure 5. *Phase contrast photographs of MCF-7 cells at 48 hours (x200)*

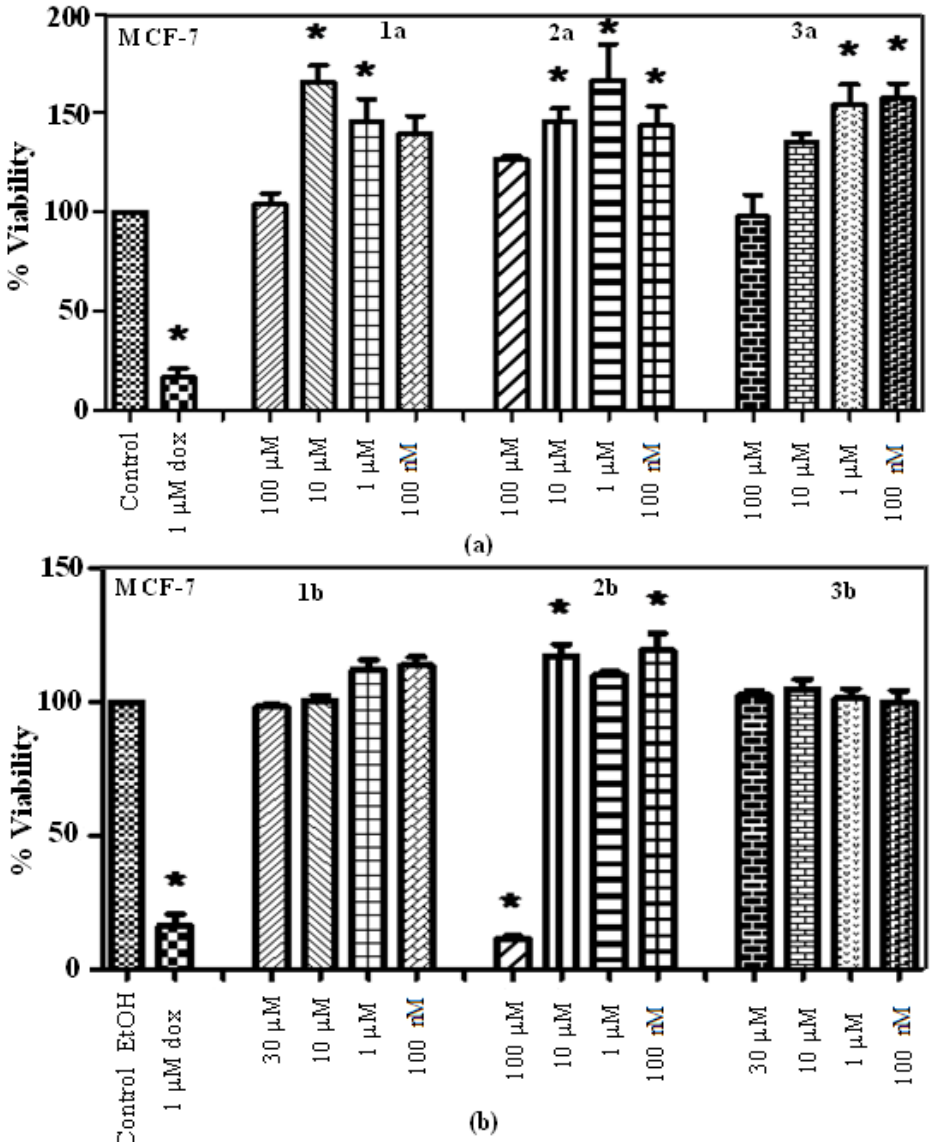

Figure 6. *The evaluation of cytotoxic activity of Schiff bases against MCF-7 carcinoma cell line; a) the monosodium-Schiff bases (**1a-3a**), b) the neutral-Schiff bases (**1b-3b**). Dox, is Doxorubicin. * shows significant difference from control group. (One-way analysis of ANOVA, Tukey, $p < 0.05$, $n = 3$)*

In order to find the side-effect of Schiff bases on normal human cell line, their cytotoxicity against HEK293 cell line was also investigated under the same conditions. Microphotographs of HEK293 cell were presented in Figure 7. After treatment of the monosodium-Schiff bases, **1a** did not show any toxicity at the concentration ≤ 10 μM (Figure 8a). Schiff base **3a** was not cytotoxic within the concentration range of 100 μM-100 nM. But, Schiff base **2a** seemed to be anomalous in series, which has weak antiproliferative effect toward HEK293 cell line in the all dose range used (100 μM-100 nM). Interestingly, the neutral-Schiff base **1b** displayed low toxicity against HEK293 above and below the concentration value of 1 μM (Figure 8b). Schiff base **2b** had lowest cytotoxic activity in the range of 100 μM-1 μM. On the other hand, Schiff base **3b** was found to be inactive between 30 μM and 100 nM. In comparison to Schiff bases, doxorubicin showed the highest cytotoxicity against HEK293 cell with the 10% viability value at the concentration of 1 μM.

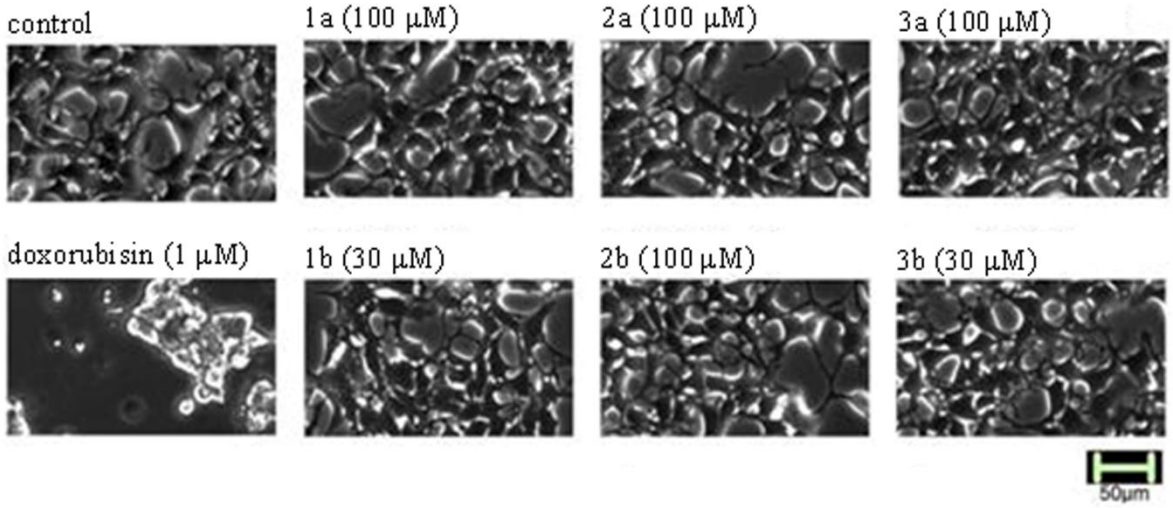

Figure 7. *Phase contrast photographs of HEK-293 cells at 48 hours (x200)*

Cytotoxicity assay results revealed that for HeLa cell, Schiff bases **1a-3a** showed little high toxicity than **1b**, but very low toxicity than doxorubicin. Schiff bases **2b** and **3b** behaved inactive in the concentration range 10-1 μM. It was appeared that Schiff bases **1a**, **3a**, **1b** and **3b** nearly have no cytotoxicity against MCF-7 cell at concentration of 100 μM, while **2a** increases cell viability in the all tested concentration range. Surprisingly, Schiff base **2b** presented very significant activity than other compounds, and killed 90 percent of MCF-7 cells at concentration of 100 μM. For a normal human cell line (HEK-293), doxorubicin could be classified as strongly cytotoxic. Despite this, Schiff bases **1a**, **3a** and **3b** were inactive, whereas the others showed a very very weak toxicity.

According to literature survey, it could be noted that:

i. Antitumor activity of the compounds is dependent on both structure and dose [48].
ii. Less work has been reported for free amino acid Schiff bases exhibiting antitumor activity. Amino acids conjugated quinazolinone-Schiff's bases containing nitro group on the phenyl ring possessed moderate activity against MCF-7 cancer cell lines (IC_{50} = 54.69–78.27 μg/mL) [9]. The IC_{50} value of Schiff base derived from alanine and 2,3-dihydroxybenzaldehyde was found to be > 100 μM on human hepatocellular carcinoma cancer (HepG2) cell line [29]. On the other hand, Cu(II) complex of Schiff base ligand derived from thiophene-2-carboxaldehyde and l-histidine showed high activity on human ovarian cancer cells (PA1) (IC_{50} = 91.42 μg/mL) compared to free ligand [49]. Novel Cd(II) complex of Schiff base obtained from 2-acetylpyridine and l-tryptophan displayed high anti-proliferative effect on MDA-MB-231 breast cancer cells (IC_{50} = 27 μmol/L), which was higher than that of cisplatin (IC_{50} = 82 μmol/L) [50].
iii. Cytotoxicity studies of Schiff bases based on higher amino acid molecules are not available in literature.

From these results, it might be suggested that Schiff base **2b** behaves to be a promising class of good MCF-7 inhibitor in literature.

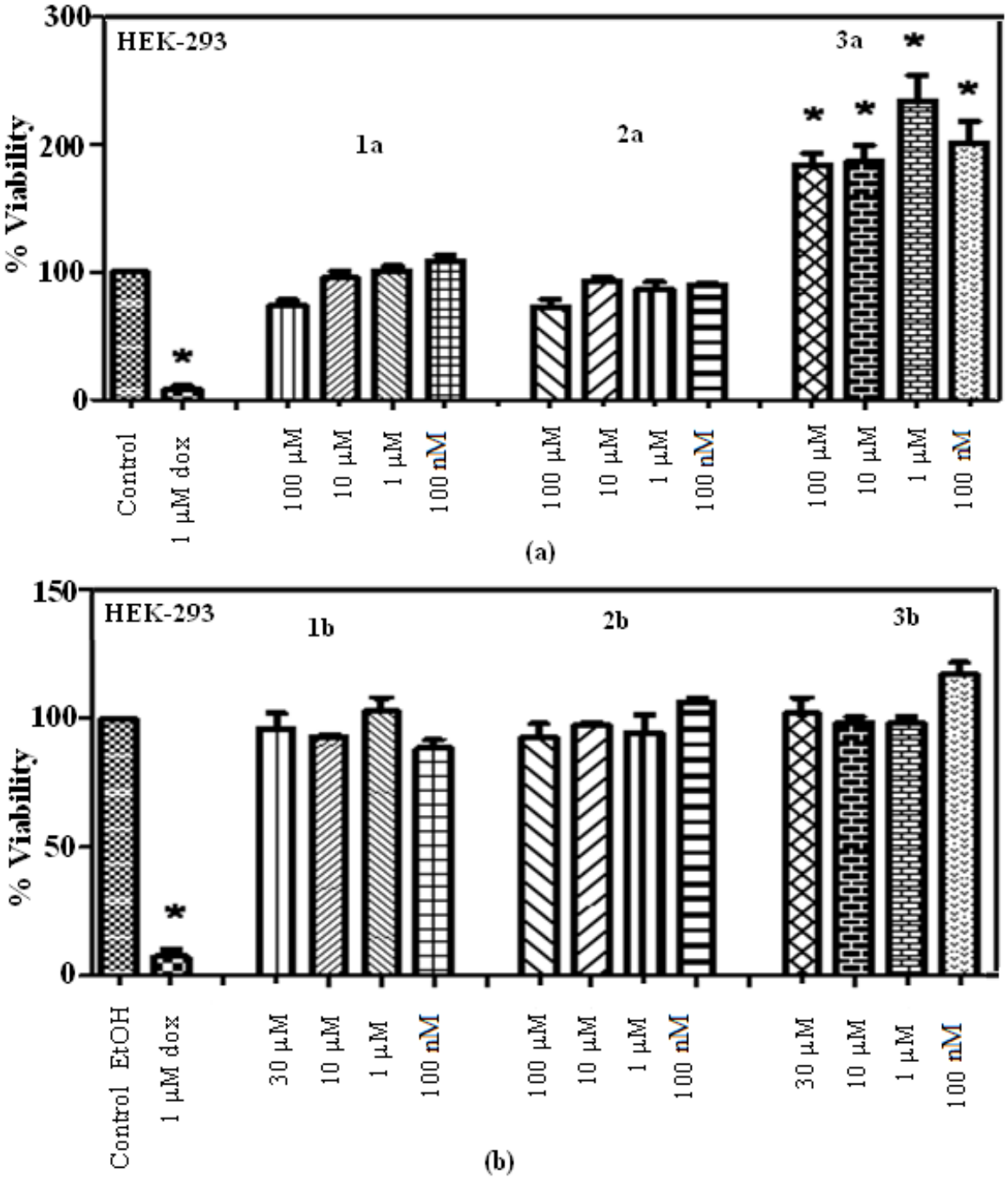

Figure 8. *The evaluation of cytotoxic activity of Schiff bases against HEK-293 normal cell line;* ***a)*** *the monosodium-Schiff bases (**1a-3a**),* ***b)*** *the neutral-Schiff bases (**1b-3b**). Dox, is Doxorubicin. * shows significant difference from control group. (One-way analysis of ANOVA, Tukey, $p < 0.05$, $n = 3$)*

4. CONCLUSION

In conclusion, the antioxidant activity of the monosodium-Schiff bases (**1a-3a**) and neutral-Schiff bases (**1b-3b**) was evaluated by DPPH assay. The results indicated that there was obvious correlation between DPPH free radical scavenging activity and the structure of ionic form of Schiff bases.

In vitro cytotoxicities of these Schiff bases exhibited that no linear relationship between cytotoxicity and structure was obtained. The presence of ionic or neutral form or increase in the number of methylene groups did not lead to increase anticancer activity.

Lastly, it was hypothesized that a good antioxidant will have better antitumor activity. Despite the fact that the neutral-Schiff bases were effective scavengers of DPPH radical, this did not lead to an increase in their cytotoxicity toward HeLa cell. The cytotoxicity results of the monosodium Schiff bases showed that their antitumor activity may be positively affected by the radical scavenging ability. But, it was found that the viability values of cancer cell were not in line with the order of SC_{50} values of the compounds. On the other hand, none of the tested compounds, except **2b,** exhibited good cytotoxicity toward MCF-7 cells. In this case, it may be suggested that no clear correlation was observed between radical scavenging and antitumor activities of these Schiff bases.

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CONFLICTS OF INTEREST

No conflict of interest was declared by the authors.

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