

## SYNTHESIS AND ANTICANCER ACTIVITY OF SOME BISQUINOXALINE DERIVATIVES

İlhan IŞIKDAĞ<sup>1\*</sup>, Yusuf ÖZKAY<sup>1</sup>, Zerrin İNCESU<sup>2</sup>

<sup>1</sup>Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry,  
26470 Eskişehir, TURKEY

<sup>2</sup>Anadolu University, Faculty of Pharmacy, Department of Biochemistry,  
26470 Eskişehir, TURKEY

### Abstract

*Resistance improvement against the existing anticancer agents creates a research area for new anticancer agents. Nevertheless, it is rather difficult to develop a new agent which can selectively inhibit the proliferation of abnormal cells with least or no affect on normal cells. Therefore, cancer chemotherapy is very important for medicinal chemists and the studies are still being carried on to develop new chemotherapeutic agents that are probable to indicate activity on various cancer types. Depending on this point, in this study some bisquinoxaline derivatives were synthesized and screened for their anticancer activity on HT-29 (colon carcinoma) and MCF-7(breast carcinoma) cell lines. Some of the synthesized compounds (**3e** and **3l**) showed significant cytotoxicity and DNA synthesis inhibition on MCF-7 cell line.*

**Key words:** Bisquinoxaline derivatives, Anticancer, Cytotoxicity, DNA synthesis inhibition.

### Bazı Biskinoksalin Türevlerinin Sentezleri ve Antikanser Aktiviteleri

*Mevcut antikanser ajanlara karşı rezistans gelişimi, yeni antikanser ajanlar için bir araştırma alanı yaratmaktadır. Bununla birlikte, normal hücreler üzerinde etki göstermeden ya da en az etkiyle anormal hücrelerin çoğalmasını seçici olarak inhibe edebilecek yeni bir ajanın geliştirilmesi oldukça güçtür. Bu nedenle, kanser kemoterapisi medisin kimyacılar için çok önemlidir ve çeşitli kanser tipleri üzerinde antikanser aktivite gösterme olasılığı bulunan yeni kemoterapötik ajanların geliştirilmesi üzerinde çalışmalar halen devam etmektedir. Bu noktadan hareketle, bu çalışmada bazı biskinoksalin türevleri sentezlenmiş ve HT-29 (kolon kanseri) ve MCF-7 (göğüs kanseri) hücre hatları üzerinde antikanser aktiviteleri için taranmıştır. Sentezlenen bileşiklerden bazıları (**3e** ve **3l**) MCF-7 hücre hattı üzerinde önemli sitotoksikite ve DNA sentez inhibisyonu göstermiştir.*

**Anahtar kelimeler:** Biskinoksalin türevleri, Antikanser, Sitotoksikite, DNA sentez inhibisyonu.

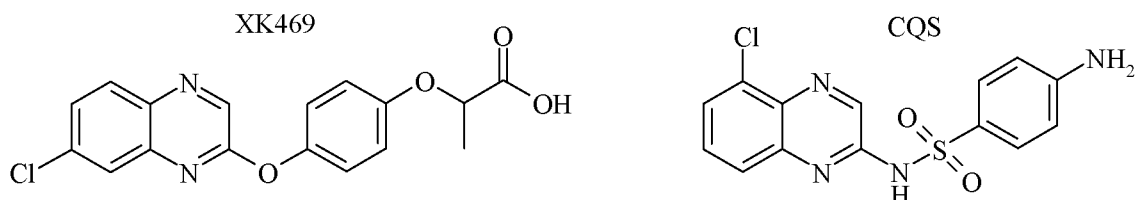
\*Correspondence: E-mail: iisikdag@anadolu.edu.tr; Tel: +90 222 3350580/3778; Fax: +90 222 3350750

## INTRODUCTION

Cancer has been a major public health threat since the beginning of the 21st century. It progresses in different organs and systems of body and has no certain etiopathology. Hence, cancer cannot be defined as a single disease and can only be described as a broad group characterized by malignant cells which are clearly distinguished from normal cells by an uncontrolled growth due to a serious disorder of the cell cycle regulatory system. Surgery, radiotherapy and immunotherapy are the methods used in the treatment of cancer. Another effective way frequently preferred for the treatment of cancer is the application of chemotherapy. Cytotoxic agents and antihormonal drugs are the main chemotherapeutics, which reduce the proliferation of malignant cells. On the other hand, significant side-effects, such as nausea, vomiting, diarrhea, hair loss, serious infections and growth of tumor-cell population are often encountered during chemotherapy (1-4).

Clinically important chemotherapeutics can be classified in three major groups. The first group is called as alkylators, which react covalently with DNA bases. DNA strand breakers constitute the second group. They are reactive radicals that produce cleavage of the polynucleotide strands. The last group includes intercalators, which insert between the base pairs of the double helix and cause a significant change of DNA conformation (5). Intercalators bind to DNA by non-covalent interactions and constitute DNA–intercalator complex. The only recognized forces that maintain the stability of the DNA–intercalator complex, even more than that of DNA alone, are van der Waals, hydrogen bonding, polarization and hydrophobic forces (5-10). The compounds, which bear heteroatoms such as nitrogen increase the strength of the complex by forming hydrogen bonds with DNA. The force of interaction between compound and DNA usually correlates with the anticancer activity (11-13). Besides, when one or more nitrogen heteroatoms exist on the chemical structure, intercalating chromophore possesses a polarized character and optimal interaction occurs (10). Based on such reasons, it has been reported that the efficiency of the stacking interactions could be enhanced with the presence of polycondensed nitrogen heterocyclic chromophore, which carries a side chain that protrude into one of the two DNA grooves (14).

Quinoxaline derivatives are double nitrogen containing heterocyclic compounds which possess biological importance. They have been reported for their application in dyes, efficient electroluminescent materials, organic semiconductors and DNA cleaving agents due to their activity against various transplantable tumors (15). For example, 2-(4-(7-chloroquinoxalin-2-yl)-phenoxy]propionic acid (XK469) (16) and chloroquinoxalinesulfonamide (CQS) (17) are the known antineoplastic quinoxaline topoisomerase II inhibitors (Scheme 1). In addition to quinoxaline derivatives, there are also some naturally occurring bisquinoxaline compounds known as antitumor antibiotics. Triostin A, azatriostin and echinomycin, which display the antitumor activity by intercalating the DNA of cancer cells, are the main examples of this antineoplastic group (18-22).



**Scheme 1.** Molecular formula of the antineoplastic quinoxalines XK469 and CQS.

Collectively, above observations and the chemotherapeutic value of the nitrogenous chemical scaffolds prompted us to synthesize some bisquinoxaline compounds. After a careful literature survey, it was revealed that syntheses of some of the designed bisquinoxalines (**3a-3c**, **3f-3j**) were reported in previous studies (23-24). On the other hand, literature survey was also showed that there has been no biological investigation for designed compounds. Hence, a study including the synthesis of some bisquinoxaline derivatives and observation of their anticancer activity was performed and reported in the present paper.

## EXPERIMENTAL

### Chemistry

Syntheses of 2,3,2',3'-tetrasubstituted-[6,6']-bisquinoxaline derivatives were performed by reacting 3,3'-diaminobenzidine (Fluka, Germany) and 1,2-dione derivatives. Acenaphthaquinone (Merck, Germany), phenanthraquinone (Fluka, Germany), diacetyl (Merck, Germany), 3,4-hexanedione (Acros, Belgium), 1,4-dibromo-2,3-butanedione (Aldrich, Germany) were the 1,2-dione derivatives purchased from a commercial chemical company. The other 1,2-dione derivatives (1,2-diaryl-ethan-1,2-diones) were prepared in our laboratory. All melting points (M.p.) were determined in open capillaries on a Gallenkamp apparatus (Weiss-Gallenkamp, Loughborough-United Kingdom) and were uncorrected. The purity of the compounds was routinely checked by thin layer chromatography (TLC) using silica gel 60G (Merck, Darmstadt-Germany). Spectroscopic data were recorded with the following instruments: IR; Shimadzu IR-435 spectrophotometer (Shimadzu, Tokyo, Japan); <sup>1</sup>H-NMR: Bruker UltraShield 500 MHz spectrometer (Bruker, Billerica, Massachusetts, USA); and Mass (Electron Spray): Agilent 1100 Series LC/MSD Trap VL&SL (Agilent, Minnesota, USA).

### General procedure for 2-hydroxy-1,2-diaryl-ethanone derivatives (1a-1g)

Appropriate 4-substitutedbenzaldehyde derivative or furan-2-carboxaldehyde (40 mmol) was dissolved in ethanol (10 ml) and sodium cyanide (0.5 g) in water (5 mL) was added. The mixture was refluxed for 2 h and then poured into ice-water. Obtained precipitate was filtered, washed with water, dried and recrystallized from ethanol.

### General procedure for 1,2-diaryl-ethan-1,2-dione derivatives (2a-2g)

Corresponding 2-hydroxy-1,2-diaryl-ethanone derivative (**1a-1g**) (10 mmol), ammonium nitrate (1.2 g) and copper (II) acetate (0.5 g) were dissolved in glacial acetic acid (20 mL) and refluxed for 3 h. Crushed ice was added into reaction mixture; precipitate was filtered, washed with water, dried and then recrystallized from ethanol.

### General procedure for 2,3,2',3'-tetrasubstituted-[6,6']-bisquinoxaline derivatives (3a-3l)

Synthesized 1,2-diaryl-ethan-1,2-dione derivative (**2a-2g**) (5 mmol) or 1,2-dione derivative (5 mmol) supplied from a commercial chemical company were dissolved in glacial acetic acid and 3,3'-diaminobenzidine (2.5 mmol, 0.5035 g) was added. Reaction mixture was refluxed for 2 h and then poured into ice-water. Precipitated product was filtered, washed with water, dried and recrystallized from glacial acetic acid.

Reaction sequence for the final products (**3a-3l**) is outlined in Scheme 2.

### 2,3,2',3'-Tetraphenyl-[6,6']-bisquinoxaline (3a)

Yield: % 86. M.p. 301-302 °C. IR (KBr)  $\nu_{\max}(\text{cm}^{-1})$  : 3055 (aromatic C-H), 1612-1388 (C=C and C=N), 696 (monosubstituted benzene). <sup>1</sup>H-NMR (500 MHz) (CDCl<sub>3</sub>)  $\delta$  (ppm): 8.60 (2H, s, quinoxaline C<sub>8</sub> protons), 8.32 (2H, d, quinoxaline C<sub>5</sub> protons), 8.24 (2H, d, quinoxaline C<sub>6</sub>

protons), 7.56 (8H, d, phenyl C<sub>2</sub> and C<sub>6</sub> protons), 7.35-7.42 (12H, m, phenyl C<sub>3</sub>, C<sub>4</sub> and C<sub>5</sub> protons). Mass (Es, m/z): M+1: 563.3.

*2,3,2',3'-Tetra-(4-methylphenyl)-[6,6']-bisquinoxaline (3b)*

Yield: % 88. M.p. 311-312 °C. IR (KBr)  $\nu_{\max}(\text{cm}^{-1})$  : 3055 (aromatic C-H), 2916 (aliphatic C-H), 1610-1383 (C=C and C=N), 821 (1,4-disubstituted benzene). <sup>1</sup>H-NMR (500 MHz) (CDCl<sub>3</sub>)  $\delta$  (ppm): 8.58 (2H, s, quinoxaline C<sub>8</sub> protons), 8.29 (2H, d, quinoxaline C<sub>5</sub> protons), 8.20 (2H, d, quinoxaline C<sub>6</sub> protons), 7.47 (8H, d, phenyl C<sub>2</sub> and C<sub>6</sub> protons), 7.18 (8H, d, phenyl C<sub>3</sub> and C<sub>5</sub> protons), 2.4 (12H, s, -CH<sub>3</sub>). Mass (Es, m/z): M+1: 619.8

*2,3,2',3'-Tetra-(4-methoxyphenyl)-[6,6']-bisquinoxaline (3c)*

Yield: % 82. M.p. 304-306 °C. IR (KBr)  $\nu_{\max}(\text{cm}^{-1})$  : 3024 (aromatic C-H), 2935 (aliphatic C-H), 1604-1378 (C=C and C=N), 835 (1,4-disubstituted benzene). <sup>1</sup>H-NMR (500 MHz) (CDCl<sub>3</sub>)  $\delta$  (ppm): 8.58 (2H, s, quinoxaline C<sub>8</sub> protons), 8.30 (2H, d, quinoxaline C<sub>5</sub> protons), 8.21 (2H, d, quinoxaline C<sub>6</sub> protons), 7.58 (8H, d, phenyl C<sub>2</sub> and C<sub>6</sub> protons), 6.93 (8H, d, phenyl C<sub>3</sub> and C<sub>5</sub> protons), 3.9 (12H, s, -OCH<sub>3</sub>). Mass (Es, m/z): M+1: 683.5

*2,3,2',3'-Tetra-(4-chlorophenyl)-[6,6']-bisquinoxaline (3d)*

Yield: % 89. M.p. 315-316 °C. IR (KBr)  $\nu_{\max}(\text{cm}^{-1})$  : 3053 (aromatic C-H), 1591-1400 (C=C and C=N), 835 (1,4-disubstituted benzene). <sup>1</sup>H-NMR (500 MHz) (CDCl<sub>3</sub>)  $\delta$  (ppm): 8.56 (2H, s, quinoxaline C<sub>8</sub> protons), 8.30 (2H, d, quinoxaline C<sub>5</sub> protons), 8.24 (2H, d, quinoxaline C<sub>6</sub> protons), 7.52 (8H, d, phenyl C<sub>2</sub> and C<sub>6</sub> protons), 7.38 (8H, d, phenyl C<sub>3</sub> and C<sub>5</sub> protons). Mass (Es, m/z): M+1: 701.2

*2,3,2',3'-Tetra-(4-isopropylphenyl)-[6,6']-bisquinoxaline (3e)*

Yield: % 81. M.p. 335-337 °C. IR (KBr)  $\nu_{\max}(\text{cm}^{-1})$  : 3047 (aromatic C-H), 2958 (aliphatic C-H), 1610-1471 (C=C and C=N), 833 (1,4-disubstituted benzene). <sup>1</sup>H-NMR (500 MHz) (CDCl<sub>3</sub>)  $\delta$  (ppm): 8.55 (2H, s, quinoxaline C<sub>8</sub> protons), 8.28 (2H, d, quinoxaline C<sub>5</sub> protons), 8.18 (2H, d, quinoxaline C<sub>6</sub> protons), 7.52 (8H, d, phenyl C<sub>2</sub> and C<sub>6</sub> protons), 7.22 (8H, d, phenyl C<sub>3</sub> and C<sub>5</sub> protons), 2.94 (4H, m, isopropyl CH), 1.28 (24H, d, isopropyl CH<sub>3</sub>). Mass (Es, m/z): M+1: 732.2

*2,3,2',3'-Tetra-(4-thiomethylphenyl)-[6,6']-bisquinoxaline (3f)*

Yield: % 83. M.p. 312-313 °C. IR (KBr)  $\nu_{\max}(\text{cm}^{-1})$  : 3055 (aromatic C-H), 2916 (aliphatic C-H), 1591-1386 (C=C and C=N), 825 (1,4-disubstituted benzene). <sup>1</sup>H-NMR (500 MHz) (CDCl<sub>3</sub>)  $\delta$  (ppm): 8.57 (2H, s, quinoxaline C<sub>8</sub> protons), 8.30 (2H, d, quinoxaline C<sub>5</sub> protons), 8.21 (2H, d, quinoxaline C<sub>6</sub> protons), 7.53 (8H, d, phenyl C<sub>2</sub> and C<sub>6</sub> protons), 7.24 (8H, d, phenyl C<sub>3</sub> and C<sub>5</sub> protons), 2.52 (12H, s, -SCH<sub>3</sub>). Mass (Es, m/z): M+1: 747.4

*2,3,2',3'-Tetra-(2-furanyl)-[6,6']-bisquinoxaline (3g)*

Yield: % 80. M.p. 292 °C. IR (KBr)  $\nu_{\max}(\text{cm}^{-1})$  : 3101 (aromatic C-H), 1614-1396 (C=C and C=N). <sup>1</sup>H-NMR (500 MHz) (CDCl<sub>3</sub>)  $\delta$  (ppm): 8.52 (2H, s, quinoxaline C<sub>8</sub> protons), 8.26 (2H, d, quinoxaline C<sub>5</sub> protons), 8.18 (2H, d, quinoxaline C<sub>6</sub> protons), 7.65 (4H, s, furan C<sub>5</sub> protons), 6.74-6.59 (8H, m, furan C<sub>3</sub> and C<sub>4</sub> protons). Mass (Es, m/z): M+1: 523.3

*11,11'-Bisphenanthro[1,2-b]quinoxaline (3h)*

Yield: % 90. M.p. 366-367 °C. IR (KBr)  $\nu_{\max}(\text{cm}^{-1})$  : 3064 (aromatic C-H), 1606-1406 (C=C and C=N). <sup>1</sup>H-NMR (500 MHz) (CDCl<sub>3</sub>)  $\delta$  (ppm): 7.81-8.32 (22H, m, quinoxaline and phenanthren protons). Mass (Es, m/z): M+1: 560.1

*9,9'-Bisacenaphtho[1,2-b]quinoxaline (3i)*

Yield: % 88. M.p. 342-344 °C. IR (KBr)  $\nu_{\max}(\text{cm}^{-1})$  : 3055 (aromatic C-H), 1612-1431 (C=C and C=N).  $^1\text{H-NMR}$  (500 MHz) ( $\text{CDCl}_3$ )  $\delta$  (ppm): 7.94-8.82 (18H, m, quinoxaline and acenaphtaquinone protons). Mass (Es, m/z): M+1: 507.3

*2,3,2',3'-Tetramethyl-[6,6']-bisquinoxaline (3j)*

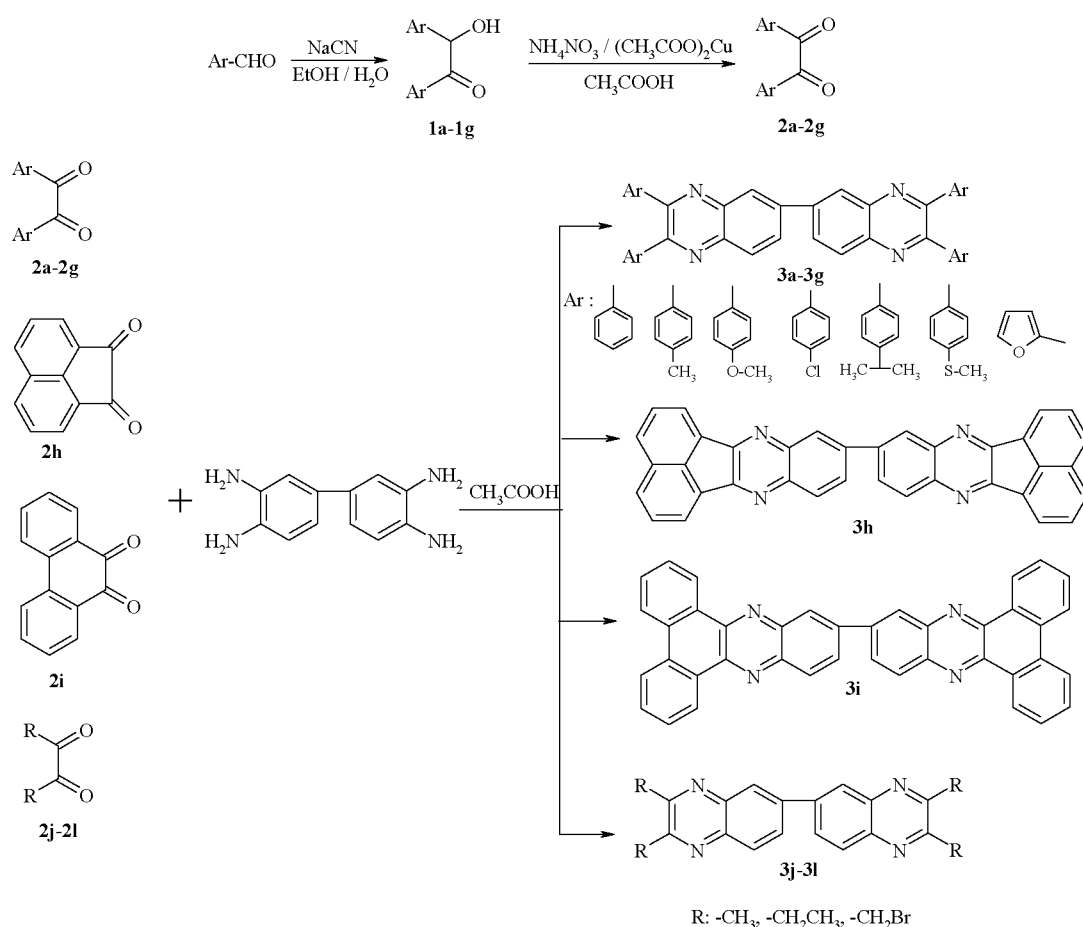
Yield: % 78. M.p. 228-229 °C. IR (KBr)  $\nu_{\max}(\text{cm}^{-1})$  : 3028 (aromatic C-H), 2914 (aliphatic C-H), 1614-1390 (C=C and C=N).  $^1\text{H-NMR}$  (500 MHz) ( $\text{CDCl}_3$ )  $\delta$  (ppm): 8.38 (2H, s, quinoxaline C<sub>8</sub> protons), 8.11 (2H, d, quinoxaline C<sub>5</sub> protons), 8.08 (2H, d, quinoxaline C<sub>6</sub> protons), 2.78 (12H, s, CH<sub>3</sub>). Mass (Es, m/z): M+1: 315.6

*2,3,2',3'-Tetraethyl-[6,6']-bisquinoxaline (3k)*

Yield: % 83. M.p. 265-267 °C. IR (KBr)  $\nu_{\max}(\text{cm}^{-1})$  : 3064 (aromatic C-H), 2972 (aliphatic C-H), 1612-1379 (C=C and C=N).  $^1\text{H-NMR}$  (500 MHz) ( $\text{CDCl}_3$ )  $\delta$  (ppm): 8.40 (2H, s, quinoxaline C<sub>8</sub> protons), 8.12 (4H, m, quinoxaline C<sub>5</sub> and C<sub>6</sub> protons), 3.09 (8H, q,  $-\text{CH}_2\text{CH}_3$ ), 1.45 (12H, t,  $-\text{CH}_2\text{CH}_3$ ). Mass (Es, m/z): M+1: 370.7

*2,3,2',3'-Tetra-(2-bromomethyl)-[6,6']-bisquinoxaline (3l)*

Yield: % 85 . M.p. 308-310 °C. IR (KBr)  $\nu_{\max}(\text{cm}^{-1})$  : 3030 (aromatic C-H), 2970 (aliphatic C-H), 1614-1419 (C=C and C=N).  $^1\text{H-NMR}$  (500 MHz) ( $\text{CDCl}_3$ )  $\delta$  (ppm): 8.44 (2H, s, quinoxaline C<sub>8</sub> protons), 8.22 (2H, s, quinoxaline C<sub>5</sub> and C<sub>6</sub> protons), 5.00 (8H, s,  $-\text{CH}_2\text{Br}$ ). Mass (Es, m/z): M+1: 631.4



**Scheme 2.** Synthesis procedure of the bisquinoxaline derivatives.

### ***Anticancer activity***

#### *Cell cultures*

MCF-7 cells were maintained in 90% Dulbecco's Modified Eagle's Medium (DMEM) (Sigma, Deisenhofen, Germany), 1 mM sodium pyruvate (Sigma, Deisenhofen, Germany), 10 µg/mL human insulin (Sigma, Deisenhofen, Germany) and 10% (v/v) of fetal bovine serum (FBS) (Gibco, U.K.). HT-29 cells were cultured in 90% McCoy's 5A (Sigma, Deisenhofen, Germany) and 10% Fetal Bovine Serum (FBS) (Gibco, U.K.). All media were supplemented with penicillin / streptomycin at 100 units/mL and cells were incubated at 37 °C in a 5% CO<sub>2</sub> / 95% air humidified atmosphere.

#### *MTT assay*

A tetrazolium salt, 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT), was used as a colorimetric substrate for measuring cytotoxicity (MTT assay). The assay was carried out according to previous study (25). Briefly, HT-29 and MCF-7 cells were cultured in 96 well plates and 1.563 to 200 µg/ml bisquinoxalines or cisplatin were added. The plates were incubated for 24 h at 37°C in 5% CO<sub>2</sub> humidified incubator together with untreated control sample. After incubation period, 20 µl MTT dye was added and the plates were measured with a ELx808-IU Bio-Tek apparatus at 540 nm. Control cell viability was regarded as 100%. Stock solutions of the test compounds were dissolved in Dimethyl sulfoxide (DMSO) and further concentrations were prepared in cell culture media. All experiments were repeated three times. For each of the compound doses, four independent wells were used. Percent viability was defined as the relative absorbance of treated versus untreated control cells.

#### *DNA synthesis inhibition assay*

DNA synthesis inhibitory effects of the synthesized compounds were performed in the 96 well flat-bottomed microtiter plates by using a BrdU colorimetric kit. HT-29 and MCF-7 cells were collected from cell cultures by 0.25% trypsin / EDTA solution and counted in a hemocytometer. Suspensions of cell lines were seeded into 96-well flat-bottomed microtiter plates at a density of  $1 \times 10^3$  cells/ mL. The tumor cell lines were cultured in the presence of various doses of the test compounds or cisplatin. Microtiter plates were incubated at 37°C in a 5% CO<sub>2</sub> / 95% air humidified atmosphere for 24 h. At the end of incubation period, the cells were labeled with 10 µl BrdU solution for 2 h and then fixed. Anti-BrdU-POD (100 µl) was added and incubated for 90 min. Finally, microtiter plates were washed with Phosphate Buffer Saline (PBS) three times and the cells were incubated with substrate solution until the colour was sufficient for photometric detection. Absorbance of the samples were measured with an ELx808-IU Bio-Tek apparatus at 492 nm. As a control solvent, DMSO was added to the cells during the time course. The values of blank wells were subtracted from each well of treated and control cells. The absorbance values of background control wells did not exceed 0.1. All experiments were repeated three times. For each dose of the compounds, triplicate wells were used.

#### *Statistics*

The SPSS for Windows 11.5 computer program was used for statistical analyses. Statistical comparison of the results obtained from controls, groups, and time periods parameters were carried out by the one-way analyses of variance (ANOVA) test and post hoc analyses of group differences were performed by using Tukey test. Results were expressed as mean ± SD.

## RESULTS AND DISCUSSION

In the present work, 2,3,2',3'-tetrasubstituted-[6,6']-bisquinoxaline derivatives (**3a-l**) were synthesized by reacting appropriate 1,2-dione derivatives (**2a-2l**) with 3,3'-diaminobenzidine. The chemical structures of the compounds **3a-l** were confirmed by IR, <sup>1</sup>H-NMR, Mass spectral data.

In the IR spectra, some significant stretching bands due to, aromatic C-H, aliphatic C-H and C=N and C=C were determined at 3101-3024 cm<sup>-1</sup>, 2972-2914 cm<sup>-1</sup>, and 1614-1378 cm<sup>-1</sup>, respectively. Stretching bands of mono and 1,4-disubstituted benzene were observed at 696 cm<sup>-1</sup> and 835-821 cm<sup>-1</sup>. In the <sup>1</sup>H NMR spectra, the signals of quinoxaline rings were clearly distinguished from the other peaks. The signal due to C<sub>8</sub> protons of quinoxalines appeared at 8.60-8.38 ppm as singlet. The signals due to C<sub>5</sub> and C<sub>6</sub> protons of quinoxalines appeared at 8.32-8.08 ppm as doublet. These <sup>1</sup>H-NMR data of quinoxaline ring are consistent with the results of previous studies (26,27). The other <sup>1</sup>H-NMR findings belonging to aromatic and aliphatic side groups were observed at expected regions. The mass spectra (Es-MS) of compounds showed [M+1] peaks, in agreement with their molecular formula.

Anticancer activity screening of the compounds **3a-3l** against HT-29 and MCF-7 cell lines was performed at two steps. In the first step, cytotoxicities of the compounds were determined by MTT method. Afterwards, DNA synthesis inhibition was analyzed for the compounds possessing significant cytotoxic activity.

In the MTT test, HT-29 and MCF-7 cell lines were incubated with the compounds **3a-3l** at various concentrations (1.563, 3.125, 6.25, 12.5, 25, 50, 100 ve 200 µg/mL). After the completion of incubation period (24 h), cytotoxic effects of the compounds were examined and IC<sub>50</sub> values were calculated. Anticancer agent cisplatin was used as a positive control. Results are presented in Table 1.

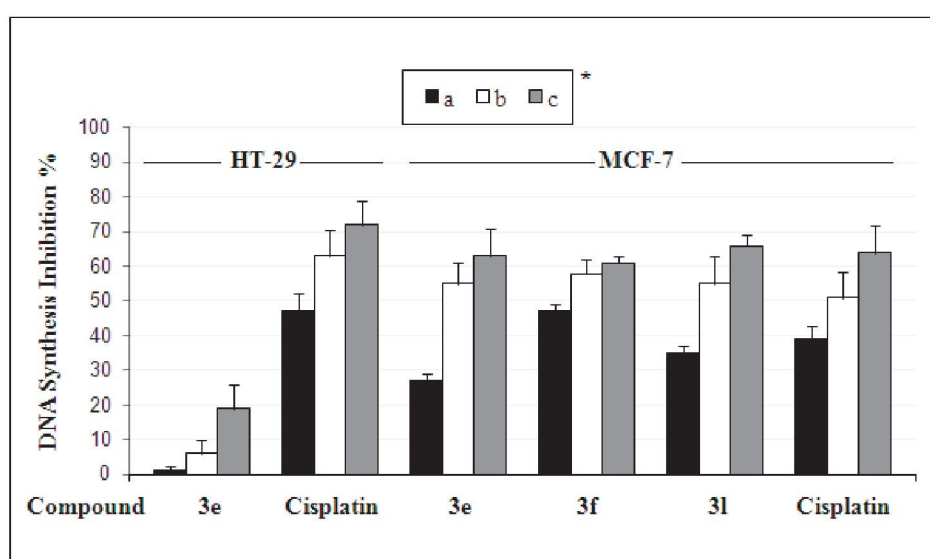
**Table 1.** IC<sub>50</sub> values of the compounds 3a-3l against the HT-29 and MCF-7 cell lines.

Compound	HT-29 IC <sub>50</sub> (µg/mL)	MCF-7 IC <sub>50</sub> (µg/mL)
<b>3a</b>	>200	>200
<b>3b</b>	>200	>200
<b>3c</b>	189.12	>200
<b>3d</b>	>200	>200
<b>3e</b>	9.37*	3.5*
<b>3f</b>	178.19	20*
<b>3g</b>	>200	>200
<b>3h</b>	>200	>200
<b>3i</b>	196.28	189
<b>3j</b>	>200	>200
<b>3k</b>	>200	>200
<b>3l</b>	142.34	5.5*
<b>Cisplatin</b>	1.7	2.6

\*Compounds found to be active against cancer cell lines and selected for DNA synthesis inhibition assay.

As seen in Table 1, the compound **3e** is the most active derivative against both of the cancer cell lines. Cytotoxic effect of this compound is very close to that of cisplatin ( $IC_{50}=2.6 \mu\text{g/mL}$ ) against MCF-7 cell line. Besides, the compounds **3f** and **3l** found to be active against MCF-7 cell lines with an  $IC_{50}$  of  $20 \mu\text{g/mL}$  and  $5.5 \mu\text{g/mL}$ , respectively. Due to their notable cytotoxicity, these compounds were chosen for DNA synthesis inhibition assay.

In the DNA synthesis inhibition assay, for 24 h time period, HT-29 and MCF-7 cells were incubated with the compounds at three different doses that were determined according to  $IC_{50}$  values. In order to make an expressive comparison with positive control cisplatin,  $IC_{50}$  and lower doses of the compounds were used. Since cisplatin possessed lower  $IC_{50}$  than the compounds, a higher dose than its  $IC_{50}$  was also preferred. Relationships between effects of the compounds on DNA synthesis and varying doses were presented in Figure 1.



**Figure 1:** Inhibitory effects of the compounds **3e**, **3f**, and **3l** on DNA synthesis of HT-29 and MCF-7 cells: mean percent of absorbance of the untreated (assessed in the presence of DMSO used as a solvent and assumed as 0%), and three different concentrations\* (on HT-29 cell line; **3e**; a:  $2.5 \mu\text{g}$ , b:  $4.5 \mu\text{g}$ , c:  $9 \mu\text{g}$ , **Cisplatin**; a:  $0.64 \mu\text{g}$ , b:  $1.7 \mu\text{g}$ , c:  $3.2 \mu\text{g}$ , on MCF-7 cell line; **3e**; a:  $0.75 \mu\text{g}$ , b:  $1.75 \mu\text{g}$ , c:  $3.5 \mu\text{g}$ , **3f**; a:  $5 \mu\text{g}$ , b:  $10 \mu\text{g}$ , c:  $20 \mu\text{g}$ , **3l**; a:  $1.375 \mu\text{g}$ , b:  $2.75 \mu\text{g}$ , c:  $5.5 \mu\text{g}$ , **Cisplatin**; a:  $0.64 \mu\text{g}$ , b:  $2.6 \mu\text{g}$ , c:  $3.2 \mu\text{g}$ ) of test compounds and cisplatin were given. Data points represent means for three independent experiments  $\pm$ SD of nine independent wells,  $p < 0.05$ .

As seen in the Figure 1, tested compounds showed dose dependent inhibitory activity on DNA synthesis of the both cell lines. The compound **3e** showed poor DNA synthesis inhibition on HT-29 cell line. However, inhibitory effect of this compound on DNA synthesis of MCF-7 cells is very remarkable. Besides, DNA synthesis inhibitory effect of the compound **3l** was very attractive against MCF-7 cell line. The highest exposed doses of the compounds **3e**, **3f**, **3l** and cisplatin were  $3.5 \mu\text{g}$ ,  $20 \mu\text{g}$ ,  $5.5 \mu\text{g}$  and  $3.2 \mu\text{g}$ , respectively. Depending on this data, it can be concluded that DNA synthesis inhibition activity of the compounds **3e** and **3f** were very close to that of cisplatin. On the other hand, although, dose of the compound **3f** was 4-6 folds higher than those of **3e** and **3l**, it could not indicate greater potency against DNA synthesis of MCF-7



cells. Thus, the compounds **3e** and **3l** were found to be more valuable than **3f** due to their significant DNA synthesis inhibitory activity at lower doses.

## CONCLUSION

The present study describes the synthesis and anticancer activity evaluation of some bisquinoxaline derivatives. Anticancer activity results showed that MCF-7 cell line is more sensitive to the bisquinoxaline derivatives. Compounds **3e**, **3f** and **3l** showed notable cytotoxicity on MCF-7 cell lines. Furthermore, in DNA synthesis inhibition assay, inhibitory activity of the compounds **3e** and **3l** was found as very close to that of cisplatin.

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