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Anticancer Drugs as a Model for Destroying and Reducing the Growth of Human Breast MDA-MB231 Cancer Cells

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ABSTRACT

Background: Azo dyes are receiving high attention in scientific research and they have great importance in chemical analysis. Cancer is a major public health problem in the world. Chemotherapy is one of the commonly-used strategies in breast cancer treatment. This therapy is usually associated with adverse side effects. The aim of current study was to produce a combination between Paracetamol and L-thyroxin drugs by synthesis of new pharmaceutical azo dye in order to reduce the growth of human breast MDA-MB231 cancer cells *in vitro*.

Method: The (*S*)-2-((4-acetamido-2-hydroxyphenyl) diazenyl)-3-(4-(4-hydroxy-3, 5-diiodophenoxy)-3,5-diiodophenyl)propanoic acid (1) was synthesised. The synthetic azo dye was derived from two different drugs (paracetamol and L-thyroxine). This azo dye was then characterized using m.p., UV-visible and IR spectrum.

Results: The synthetic azo dye provided non-toxic effects using different concentrations and it didn't show any haemolytic effect in the cells. Furthermore, the cell viability (cytotoxicity) assay presented the ability of azo dye in destroying and reducing the growth of human breast MDA-MB231 cancer cells.

Conclusion: The synthetic azo dye may be useful as a novel anticancer drug.

Keywords: *Azo dye, Cytotoxicity assay, Human breast cancer cells, Cell viability, conformational analysis.*

Introduction

Azo dyes are receiving high attention in scientific research [1,2,3,4] and they have great importance in chemical analysis. A strongly coloured compound can be yellow, red, orange, blue or even green depending on the exact structure of the molecule due to make azo dyes extremely important as dyes and also as pigments for a long time [5]. The structural features in organic compounds, that usually produce colour, are C=C, N=O, N=N, aromatic rings, C=O and NO_2. However, the groups that invariably confer colour are the azo (–N=N–) and nitroso (–N=O), while other groups actually do so under certain circumstances [6]. Azo dyes contain one or more azo groups (–N=N–) which are linked to SP_2 hybridized carbon atoms, based on the number of such groups [6]. These compounds contain more than one active group, which is able to formulate chelatic coordinational complexes with metal ions distinguished by their colour and ability to dissolve in different solvents [6]. Further, the azo is reactive compound [5] that was reported for its pharmaceutical importance as antidiabetic [6], antineoplastic [6], antibacterial [5,6] and anticancer agent [6].

Cancer is a major public health problem in the world [6]. Chemotherapy is one of the commonly-used strategies in breast cancer treatment. This therapy is usually associated with adverse side effects [10] ranging from nausea to bone marrow failure [6] and development of multidrug resistance (MDR) [6]. Cytotoxicity has been defined as the cell-killing property of a chemical

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compound independent from the mechanism of death [6]. Cytotoxicity assay is an appropriate method for screening new substances within a short time in order to determine their cytotoxicity on cancer cells [6]. Usually in oncology research and clinical practices, *in vitro* testing is preferred prior to *in vivo* testing. The *in vitro* cultures can be refined under controlled environmental conditions (pH, temperature, humidity, oxygen/CO_2 balance etc.) resulting in homogenous batches of cells and thus minimizing experimental errors [16]. The aim of current study was to makre a combination between the paracetamol and L-thyroxin drugs by synthesis of new pharmaceutical azo dye in order to reduce the growth of human breast MDA-MB231 cancer cells *in vitro*.

Method

The melting point of the azo dye was attended using Buchi B190K. The IR spectrum was carried out on a FT-IR-8400S. Fourier Transform Infrared Spectrophotometer Shimadzu (Japan) by using a KBr disc in the range 500–4000cm^{-1}. Absorption spectrum in ethanol with the concentration of ($1x10^{-4}$M) was determined on a spectrophotometer. The IR, UV-Visible spectrophotometer and the melting point were performed in the Chemistry Department/College of Education for pure science/University of Basra, Iraq.

Synthesis of (S)-2-((4-acetamido-2-hydroxyphenyl) diazenyl)-3-(4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl)propanoic acid: This azo dye was synthesised by a method similar to that nominated by Fox [17]. It was prepared by dissolving paracetamol (0.006mol; 0.907g) in concentrated HCl (2.1mL) followed by adding 10mL of distilled water with continuous stirring in ice bath to keep the temperature below (-5°C). Also, the $NaNO_2$ was prepared by dissolving 0.468g in 5mL of distilled water and then was added to the first solution drop by drop with keeping the temperature below (-5°C). The resulting solution was then added to the L-thyroxine (0.006mol; 4.661g) in 25% NaOH. The resulting crudes were recrystallized in ethanol and hexane to yield the titled azo dye (3.5g; 63%)mp showed decomposition of azo dye; ν_{max}: 3327.21, 3302.13, 3207.62, 3041.74, 1662.64 and 1375.25cm^{-1}.

Solution of azo dye in ethanol: The solution of the azo dye was prepared by dissolving ethanol to give ($1x10^{-4}$M) concentration.

Cellular toxicity: The Xian-guo and Ursola method [6] was applied to measure the toxity of azo using hemolytic red blood cells as follows: A stock solution of 200mg/mL was prepared and followed by preparing a series of diluted (0.2, 0.3 and 0.4mg/mL) solutions. Then 0.8mL of each diluted solution was added to Eppendorf tubes and 0.2mL of red blood cells was also added to each tube. In addition, two Eppendorf tubes were equipped; in the first tube, 0.8mL of Ringer solution was added as a negative control, but the tap water as a positive control was added to second tube. Then 0.2mL of red blood cells was added to each tube. The results were recorded after the incubation of these tubes for 37 minutes in a special incubator and the changes in the solutions were checked.

Cell culture: Human breast cancer MDA-MB231 cells were maintained in 10cm plate contained DMEM supplemented with 10% FBS, 100units/ml penicillin and 100µg/ml/ml streptomycin at 37°C and humidified atmosphere with 5% CO_2.

Cytotoxicity: The MDA-MB231 cells were grown in 96-well plate for 24h and then treated with 100µM of the prepared azo dye for 24h. Cells viability was measure at 570nm in a micro-plate reader (Thermo Scientific) and the experiment was repeated 3 times. The results were measured in Salman Abad University in Pakistan.

Result and Discussions

The azo dye, named (*S*)-2-((4-acetamido-2-hydroxyphenyl) diazenyl)-3-(4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl)propanoic acid, was synthesised [17] (Figure 1).

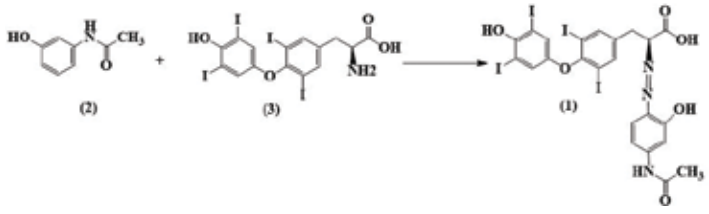


Figure 1: Synthesis of the synthetic azo dye

This azo dye was derived from two different drugs (paracetamol and L-thyroxine 3) using a method similar to that designated by Fox [17] with optimizing the stoichiometry and the conditions of the reaction. Thyroxine [6] was the first hormone replacement therapy, first initiated more than a century ago. Its absorption after ingestion is largely in jejunum and ileum. The absorption is affected by many pharmacological agents and herbal remedies. Levothyroxine is a commonly prescribed thyroid medication that is used to treat hypothyroidism and goiter [6]. A previous study mentioned that oxidative stress could be increased using levothyroxine medication, because it leads to the overproduction of reactive oxygen species (ROS) in the body. Due to the hypermetabolic status, increased oxidative stress induces chronic inflammation. However, some studies also confirmed that chronic inflammation is usually connected to some chronic conditions such as cancer, diabetes, and heart disease [19]. As human cell structure and function could be altered owing to overproduction of ROS; hence, imbalance of body function may induce somatic mutations and neoplastic transformation. In addition, oxidative stress has been linked to cancer initiation and progression, because it promotes DNA damage and cell proliferation by increasing DNA mutation. A recent study has shown that activation of phosphoinositol 3-kinase (PI3K) and the mitogen-activated protein kinase (MAPK) due to the effect of thyroid hormone are responsible for breast cancer cell proliferation. Indeed, thyroxine can activate these pathways and promote breast cell proliferation [19]. Though paracetamol/acetaminophen is one of the most popular and most commonly used analgesic and antipyretic drugs around the world, it is available without prescription, both in mono- and multi-component preparations [6]. It is the drug of choice in patients who cannot be treated with non-steroidal anti-inflammatory drugs (NSAID) such as people with bronchial asthma, peptic ulcer disease, haemophilia, salicylate-sensitized people and children under 12 years of age, pregnant or breast-feeding women. It is recommended as a first-line treatment of pain associated with osteoarthritis. The mechanism of action is complex and includes the effects of both peripheral (COX inhibition), and central (COX, serotonergic descending neuronal pathway, L-arginine/ NO pathway, cannabinoid system) antinociception processes and redox mechanisms. The current study was focused on combination between the paracetamol and L-thyroxin drugs by synthesis of new pharmaceutical azo dye in order to reduce the growth of human breast MDA-MB231 cancer cells *in vitro*.

The synthetic azo dye was then characterized using m.p. (which showed that the azo dye was decomposed), UV-visible and IR spectrum. The UV-visible spectrum was documented at the range 250-450nm. The results showed that the maximum wave length of the azo dye was equal to 260nm and 380 and 430nm related to (π -π*) and (n-π*), respectively, as shown in Figure (2).

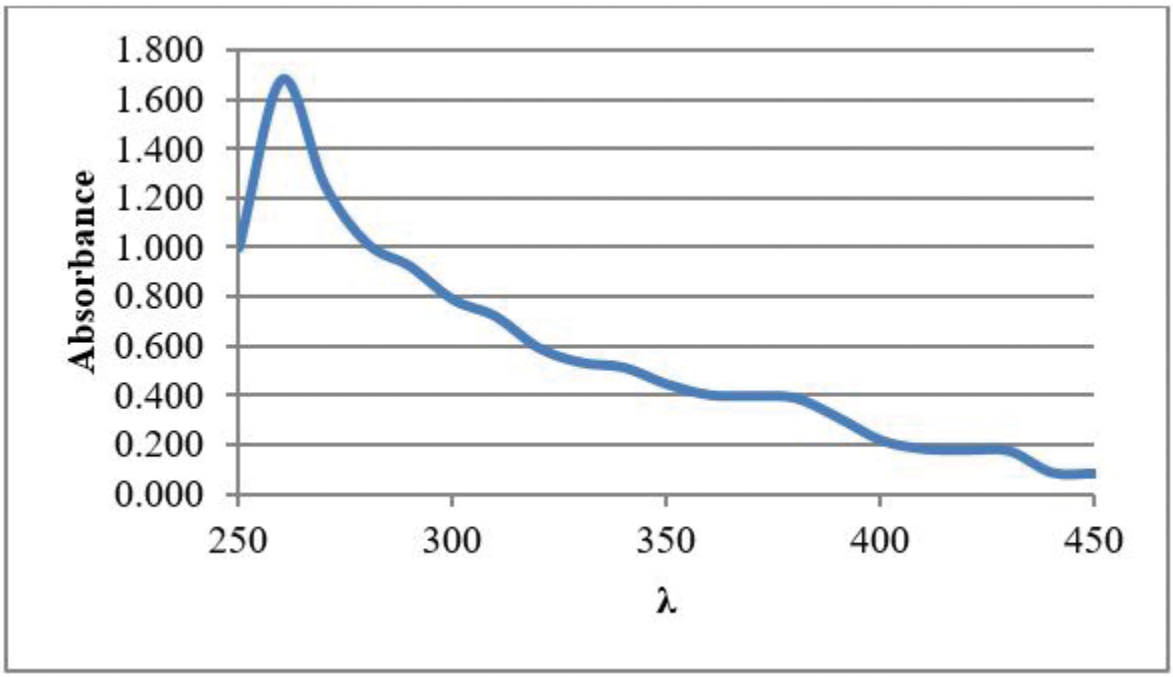


Figure 2: The UV-Vis spectrum of the synthetic azo dye

The IR spectrum of the synthetic azo dye (Figure 3) was also studied. The results showed that the stretching vibration of the ν (OH) group appeared in the region 3327.21cm^{-1}.

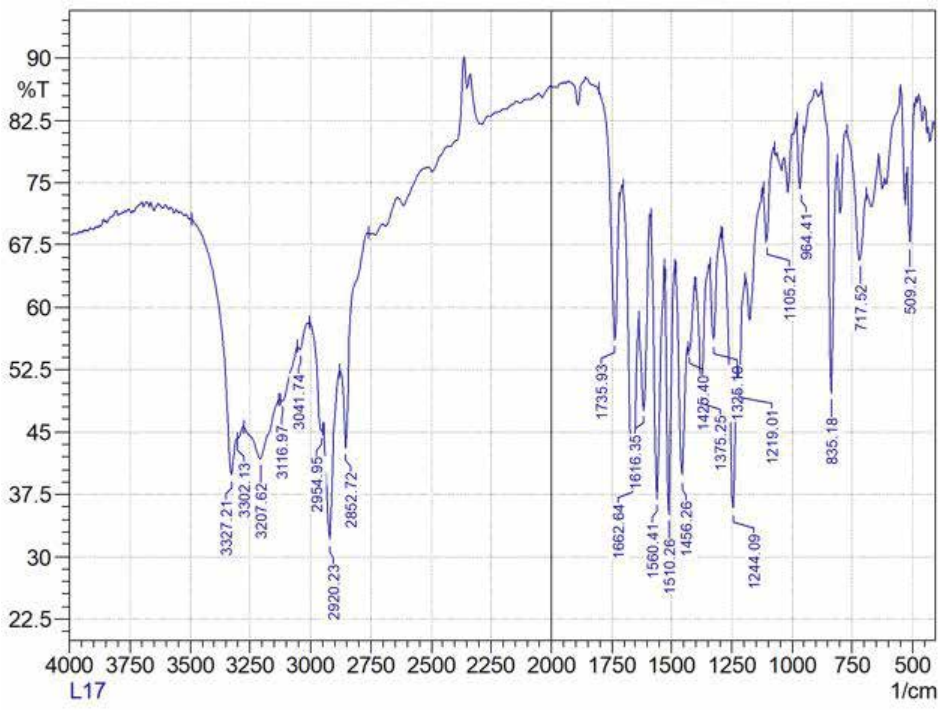
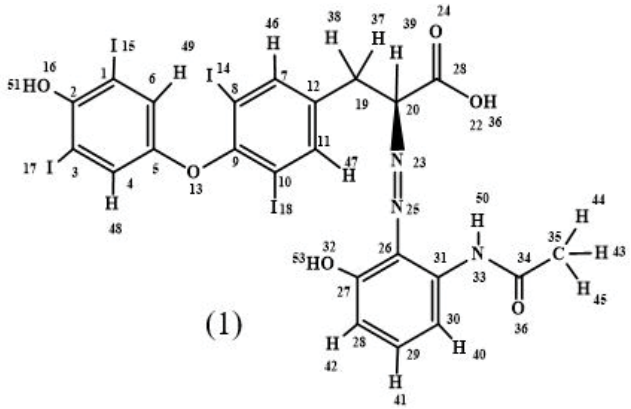


Figure 3: IR spectrum of the synthetic azo dye

But, the ν (N=N) stretching vibration band seemed in the region 1456.26cm^{-1} [2,3]. Other bands with this region can be considered as skeletal vibrations. The (C=C) stretching vibration of the aromatic ring showed a strong band in the region 1510.26 cm^{-1} [2,3] and the aromatic CH band appeared in the region 3041.74cm^{-1} [3].

The method of [13] was then applied to measure the toxicity of the synthesised dye using haemolysis of red blood cells *in vitro*. The results showed that the azo dye provided non-toxic effect and didn't induce any haemolytic effect despite using different concentrations. The cell viability (cytotoxicity assay) was used for to observe its (azo dye) ability in destroying live cancer cells and reducing living cells growth for human breast MDA-MB231 cancer cells. After 24h treatment with 100µM, the results were presented in Table (1) below.

Table 1: Values of the cell viability test after 24h of treatment with 100µM of the synthetic azo dye

Sample	Viability % 1	Viability % 2	Viability % 3
 (1)	42.07048	51.80723	62.25403
Control	100	100	100

The results of the cytotoxicity assay revealed high cytotoxic action of the azo dye on the viability of cancer cells in contrast to the control. In addition, the average of this effect was 52.04391, which indicated that the action of the azo dye was around 50% in contrast to the 100% action in the controls.

Theoretical studies were performed on the synthetic azo dye to understand the effect of its structure in its anticancer activity. Conformational analysis of the azo dye in question was also studied theoretically. The results showed that the conformational energy around single bond in each side of azo group was generating eclipsed and staggered conformers. The energy of C(26)-C(27)-N(25)-N(23) and N(25)-N(23)-C(20)-C(19) conformers were presented in Figure (4a and b) below.

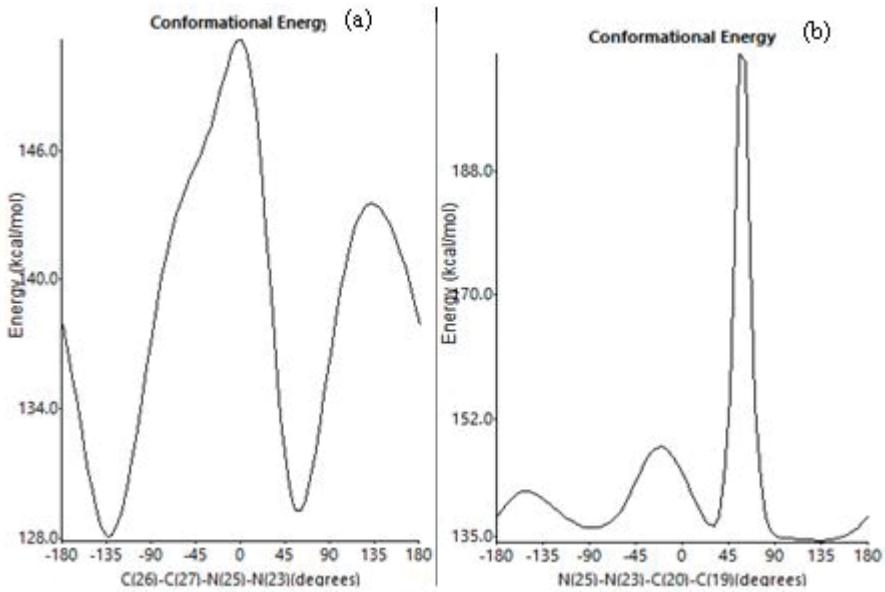


Figure 4: The conformational energy of each C(26)-C(27)-N(25)-N(23) (a) and N(25)-N(23)-C(20)-C(19) (b) conformers in the synthetic azo dye

The results showed that each rotation around each side can generate eclipsed and staggered conformers as seen in Figure (4) above. Figure (4a) showed that the eclipsed E for E(-180°), E(0°) and E(135°) were equal to 137.89, 151.19 and 143.54kcal/mole, respectively. However, the staggered conformers for E(-130°), E(6°) and E(180°) were equal to 128.07, 129.26 and 137.89kcal/mole, respectively. On the other hand, Figure (4b) showed that the eclipsed E for E(-150°), E(-20°), E(55°) and E(180°) were equal to 141.48, 147.92, 205.08 and 137.89kcal/mole, respectively. In addition, the staggered conformers for E(-180°), E(-85°), E(30°) and E(135°) were equal to 137.89, 136.17, 136.47 and 134.35kcal/mole, respectively.

Furthermore, the MM2 properties were envisioned for the azo dye of interest, the results revealed that the stretch, cubic stretch, quartic stretch, bend, stretch-bend, torsion, non-1,4 VDW, 1,4 VDW, dipole/dipole and the total energy were equal to 37.0880, -2.0000, 2.3330, 20.2607, -0.3695, 29.3927, -5.2310, 1.3248, 1.6257 and 84.0915kcal/mol, respectively. When comparing these results with those received by anticancer activity, we found that the azo dye gave better ability in destroying living cancer cells. Therefore, the non-toxic azo dye can be used as a novel anti-cancer drug. Further, the MM2 minimization for the azo dye was studied and data from current study showed that the stretch, bend, stretch-bend, torsion, non-1,4 VDW, 1,4 VDW, dipole/dipole and the total energy were equal to 1.9484, 11.0444, 0.1601, -20.4440, -11.5854, 24.0501, -3.4969 and 1.6767kcal/mol, respectively. The MMFF94 total energy, MMFF94 minimization iteration energy and MMFF94 minimization/sampling energy were equal to 131.660, 80.0929 and 79.8793kcal/mol, respectively. All these results showed that the minimization was attended successfully.

Conclusion

The azo dye was cheap to prepare, because the starting materials are available and most of the chemistry is completed at or below room temperature. Also, the synthetic azo dye gained good colour, delivered non-toxic effect and didn't show any haemolytic effect in the red blood cells. Furthermore, the azo dye had good ability to bind breast cancer MDA-MB231 cells and affect their viability. The molecular mechanics calculated the energy of a molecule and then adjusted the energy through changes in bond lengths and angles to obtain the minimum energy structure. The rotation of atoms about the single bonds is the subject of conformational analysis. This can affect the internal coordination because it affects the binding of molecules and can make this azo dye novel anti-cancer drug.

Ethical Clearance: The research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

Conflict of Interest: The authors declare that they have no conflict of interest.

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