Preparation of novel Azo Dyes as a new anti-Human Breast Cancer MDA-MB231 Cells and study its association with DNA

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Abstract

The 4,6-*bis*((*E*)-(3-nitrophenyl)diazenyl) benzene-1,3-diol (**1**), 4-((*E*)-(3-nitrophenyl)diazenyl)-6-((*E*)-(4-nitrophenyl)diazenyl)benzene -1,3-diol (**2**), 4-((*E*)-(2-nitrophenyl) diazenyl)-6-((*E*)-(3-nitrophenyl)diazenyl)benzene-1,3-diol (**3**), 4,6-*bis*((*E*)-(2-nitrophenyl) diazenyl)benzene-1,3-diol (**4**), (*N*-(4-hydroxy-3-(((4-(5-(*p*-tolyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl)phenyl)sulfonyl)diazenyl)phenyl)acetamide (**5**), 3-(isopropylamino)-1-(4-(2-methoxyethyl)phenoxy)-1-((3-nitrophenyl)diazenyl)propan-2-ol (**6**), 3-(isopropylamino)-1-(4-(2-methoxyethyl)phenoxy)-1-((2-nitrophenyl)diazenyl)propan-2-ol (**7**) and 4-(((4-(5-(*p*-tolyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl)phenyl)sulfonyl) diazenyl)benzene-1,3-diol (**8**) were synthesised. The resulting diazo dyes and pharmaceutical azo dyes were then characterized using (m.p., IR, UV-visible and mass spectrum) and (m.p., IR, UV-visible and ^{1}H NMR spectrum) respectively. Add to which, all the synthetic azo dyes were provided non-toxic effects using different concentrations from each, and didn't show any hemolysis effect in the cells. The cell viability (cytotoxicity assay) is used for each dye to observe their ability in destroying cancer living cells. And reduce its growth for human breast MDA-MB231 cancer cells, after 24h treatment with 100µM of each dye. The results were showed well activities of each dye against cell viability in contrast with the control. Further, the results of human DNA binding of each azo dye were indicated its ability to damage DNA and inhibiting of DNA transcription and replication.These results with that obtained by the NanoDrop™ spectrophotometer were showed increasing in the concentration of the nitrogen bases, which confirmed the DNA was damaged. Due to recommend the synthetic, non-toxic azo dyes as novel drugs for treatment of human breast MDA-MB231 cancer cells through its ability to destroy the DNA of the cancer cells.

Key words: Azo dyes, Cytotoxicity assay, Human breast cancer, Cell viability, Transcription, Replication

1. Introduction

Azo compounds were 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 as extremely importance as dyes and also as pigments for a long time.[5] The structural features in the organic compounds, that usually produce colour are C=C, N=O, N=N, aromatic rings, C=O and NO_2. Though, the groups that invariably confer colour are the azo (–N=N–) and nitroso (–N=O), while the 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.[7] These compounds contain more than one active group, which is able to formulate chelatic coordinational complexes with metal ions distinguished by their color and ability to dissolve in different solvents.[6] Further, the azoisreactivecompound,[5]that was reported for its pharmaceutical importance as antidiabetic,[8] antineoplastic,[9] antibacterial,[5,10] and anticancer agent.[11]Add to which, the azo molecules are known to be involved in the inhibition of DNA, RNA, carcinogenesis, and protein synthesis. The presence of -N=N- in the molecular structure of azo is responsible for the interaction with the active site of protein.[11]

Cancer is a major public health problem in the world.[12] Chemotherapy is one of the commonly-used strategies in breast cancer treatment. This therapy is usually related with adverse side effects,[10] ranging from nausea to bone marrow failure[13] and development of multidrug resistance (MDR).[14]Cytotoxicity has been defined as the cellkilling property of a chemical compoundindependent from the mechanism of death.[15] Cytotoxicity assay is an appropriate method forscreening new substance within a shorttime in order to determine cytotoxicity oncancer cells.[16] Usually inoncology research and clinical practices, in vitro testing is preferred prior to in vivotesting. In vitro cultures can be refined under a controlled environment (pH, temperature, humidity, oxygen/ CO_2balance etc.) resulting in homogenous batches of cells and thus minimizing experimental errors.[16]

2. Experimental section

The melting points of diazo dyes and the pharmaceutical azo dyeswere attended usingBuchi B190K. The IR spectrum was carried out on aFT-IR-8400S.Fourier Transform Infrared Spectrophotometer Shimadzu (Japan) by using a KBr disc in the range (600 – 4000) cm^{-1}. Absorption spectrum in ethanol with the concentration of (1 x 10^{-4} M) was determined on a spectrophotometer. The IR, UV-Visible spectrophotometer and melting point were performed by Chemistry Department– Education College of pure science– Basrah University, Iraq.Accurate mass spectrumand the ^1H NMR were measured in Tehran University in Iran.

2.1 Synthesis of diazo dyes

The diazo dyes, (1), (2), (3) and (4) were synthesised by a method similar to that designated by Fox[17].Each di azo dye was synthesised Using x-nitroaniline (0.006 mol., 0.828 g), (9), (10) and (11) respectively and $NaNO_2$ (0.468 g) in 2.1 mL of diluted HCl, (x= ortho- NO_2, meta- NO_2 and para- NO_2).The eachresulting diazonium salt was then added to x-nitro azo dye[18](0.006 mol., 1.555 g), of each (12), (13) and (14)in 25% sodium hydroxide solution to yield (2.211 g, 90%), (1.969 g, 80%), (1.889 g, 77%) and (1.429g, 58%) from (1), (2), (3) and (4) respectively. The resulting crudes were recrystallized in ethanol and hexane to yield light to dark red brown compounds; m.p.(–183184) ^oC, (184 – 185) ^oC, (187 – 188) ^oC and (189–190) ^oC respectively; ν_{max}: (3429.43, 3089.96, 1514.12 and 1444.68) cm^{-1} (1), (3466.03, 3088.03,1525.69 and 1431.18)cm^{-1} (2), (3464.15, 3089.96, 1525.69 and 1450.47) cm^{-1}(3) and (3468.37, 3107.30, 1521.84

and 1448.54) cm^{-1}(4); λ_{max}:(290, 360) nm, (290, 390) nm, (280, 370) nm and (280, 360) nm. The accurate mass value of each di azo dye was equal to408.6.

2.2Synthesis of pharmaceutical azo dyes

The azo dyes, (5), (6), (7) and (8) were also synthesised by a method similar to that nominated by Fox[17]. The azo dyes were prepared using each of celecoxib (15), m-nitro aniline (10) and o-nitro aniline (9), using (0.006 mol., 0.828g), (0.006 mol., 2.288 g), (0.006 mol., 0.828g) respectively and with (2.1 mL) conc. HCl in separate beakers followed by add 10 mL of distilled water to each. Then, the $NaNO_2$ were prepared by dissolving 0.468 g four times in 5 mL of distilled water, each $NaNO_2$ solution was then added to each beaker. The resulting diazonium salts was then added to paracetamol (16), metoprolol (17) and resorcinol (18) using (0.006 mol., 0.907 g), ((0.006 mol., 1.604 g) and (0.006 mol., 1.604 g)) and (0.006 mol., 0.661 g) respectively in 25% sodium hydroxide solution to yield (2.700 g, 83%), (1.991 g, 80%), (2.151 g, 86%), and (2.700 g, 90%) respectively. The resulting crudes were recrystallized in ethanol and hexane to yield the titled azo dyes; m.p.: (146–147) ^oC, (69–70) ^oC, (99–100) ^oC and (88 – 99) ^oC respectively; This showed δ_H (500 MHz, DMSO): 9.68 (2H, s), 7.88 (2H, d, *J* 9.9 Hz), 7.54 (2H, d, *J* 9.9 Hz), 7.25-7.20 (2H, m), 7.18 (1H, s), 6.67(3H, d, *J* 9.9 Hz), 6.22-6.18 (2H, m), 3.35 (3H, s), 2.13(3H, s) (**1**), 8.00 (1H, br s), 7.93 (1H, d, *J* 9.9 Hz), 7.67 (1H, d, *J* 9.9 Hz), 7.57 (1H, t, *J* 4.99 Hz), 7.13 (2H, d, *J* 9.9 Hz), 6.85(2H, d, *J* 9.9 Hz), 5.34 (1H, s), 4.30 (1H, s), 4.05 (1H, dd, *J* 4.9, 14.9 Hz), 3.67 (1H, dd, *J* 4.9, 9.9 Hz), 3.46 (2H, t, *J* 9.9 Hz), 3.38-3.28 (2H, m), 3.24-3.18 (3H, m), 1.40-1.30 (2H, m) (**2**); 7.75 (1H, d, *J* 9.9 Hz), 7.57 (1H, t, *J* 14.9 Hz), 7.50 (1H, d, *J* 9.9 Hz), 7.27 (1H, t, *J* 4.99 Hz), 7.11 (2H, d, *J* 4.9 Hz), 6.85 (2H, d, *J* 9.9 Hz), 5.55-5.25 (1H, m), 4.20 (1H, br s), 4.10-4.08 (1H, m), 4.00 (1H, dd, *J* 4.9, 14.9 Hz), 3.85 (3H, dd, *J* 4.9, 14.9 Hz), 3.74 (3H, br s), 3.55 (1H, m), 3.40-3.30 (2H, m), 2.73-2.70 (1H, m), 1.36-1.32 (2H, m) (**3**); 9.13 (2H, s), 7.88 (2H, d, *J* 9.9 Hz), 7.51-7.55 (2H, m), 7.18-7.22 (3H, m), 7.15 (1H, s), 6.91 (2H, t, *J* 9.9 Hz), 6.22-6.18 (2H, m), 2.5(3H, s) (4); ν_{max}:(3338.78, 3099.61, 1600.00, 1350.00 and 1348.24)cm^{-1} (1), (3425.58, 3066.11, 1612.49, 1456.26 and 1346.31)cm^{-1} (2), (3433.29, 3000.00, 1637.55, 1527.62 and 1355.96) cm^{-1}(3) and (3255.84, 3226.91, 2924.09, 1608.63, 1379.10 and 1300.02)cm^{-1} (4); λ_{max}: (280, 350) nm, (280, 380) nm, (280, 400) nm and (270, 310) nm.

2.3 Solution of diazo dyes and the pharmaceutical azo dyes in ethanol

The solutions of diazo dyes and the pharmaceutical azo dyes were prepared by dissolved in ethanol to give ($1x10^{-4}$ M) concentration.

2.4Cellular toxicity

The Xian-guo and Ursola method[19] was applied to measure the toxity of diazo dyesand thepharmaceutical azo dyes under study, usinghemolytic red blood cells as following: A stock solution of 200 mg / mL was prepared and followed by preparing a series of diluted (0.2, 0.3 and 0.4 mg/ mL) solutions. 0.8 mL of each diluted solution was added to Eppendorf tubes. 0.2 mL of red blood cells was also added to each tube. In addition, two Eppendorf tubes were equipped. In the first tube, 0.8 mL of Ringer solution was

added as a negative control, but the tap water as a positive control was added to second tube. Then 0.2 mL 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 followed checked.

2.5 Cell culture

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

2.6 Cytotoxicity

The MDA-MB231 cells were growth in 96 well/ plate for 24 h and then treated with 100µM of each dye (1-8) for 24h. Cell viability was measure at 570 nm in a micro-plate reader (Thermo Scientific), and the experiment repeated 3 times.The results were measured in Salman Abad University in Pakistan.

2.7Human Genomic DNA extractions

Nucleic acids from each 200 µL of EDTA-whole blood sample were extracted. After cell lysis and protein denaturation, according to the procedure of Sambrook *et al.*[20] and stored frozen until use.

2.8Effect of diazo dyes and the pharmaceutical azo dyes on human DNA

To study the effect of azo dyes (1-8) on genomic human DNA, 10 µl. of each was mixed with 10 µl of human genomic DNA, the mixture incubated at 37 ºC, and then subjected to 0.8% agarose gel electrophoresis at 60V. The DNA concentrations before and after binding with each synthetic azo dyes (1-8) were measured using a NanoDrop™ spectrophotometer at A 260 nm.

Result and dissection

The azo dyes that characterised4,6-*bis*((*E*)-(3-nitrophenyl)diazenyl)benzene-1,3-diol (**1**), 4-((*E*)-(3-nitrophenyl)diazenyl)-6-((*E*)-(4-nitrophenyl)diazenyl)benzene-1,3-diol (**2**), 4-((*E*)-(2-nitrophenyl)diazenyl)-6-((*E*)-(3-nitrophenyl)diazenyl)benzene-1,3-diol (**3**) and4,6-*bis*((*E*)-(2-nitrophenyl) diazenyl)benzene-1,3-diol (**4**), (*N*-(4-hydroxy-3-(((4-(5-(p-tolyl)-3-(tri fluoro methyl)-1H-pyrazol-1-yl) phenyl) sulfonyl)diazenyl)phenyl) acetamide (**5**), 3-(isopropyl amino)-1-(4-(2-methoxyethyl) phenoxy)-1-((3-nitrophenyl)diazenyl) propan-2-ol (**6**), 3-(isopropyl amino)-1-(4-(2-methoxyethyl) phenoxy)-1-((2-nitro phenyl)diazenyl) propan-2-ol (**7**) and 4-(((4-(5-(p-tolyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl)phenyl)sulfonyl) diazenyl) benzene-1,3-diol (**8**)) were synthesised, (Figures1-8).

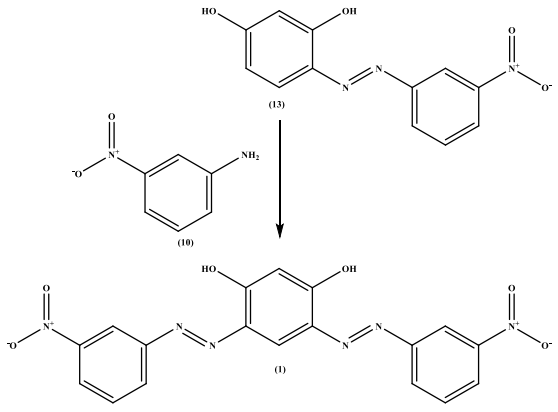


Figure (1): Synthesis of (1).

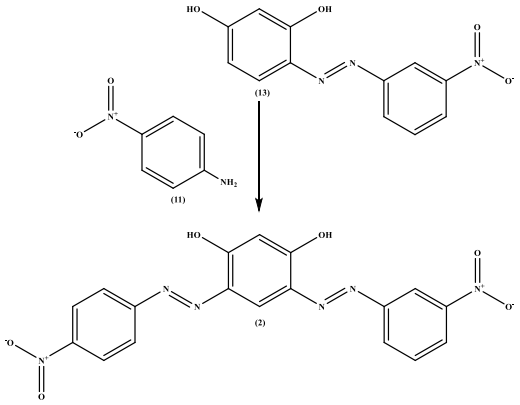


Figure (2): Synthesis of (2).

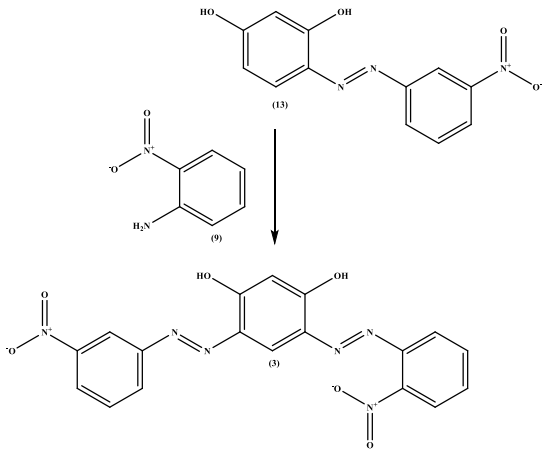


Figure (3): Synthesis of (3).

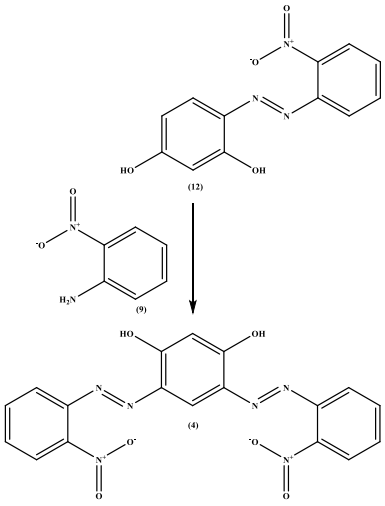


Figure (4): Synthesis of (4).

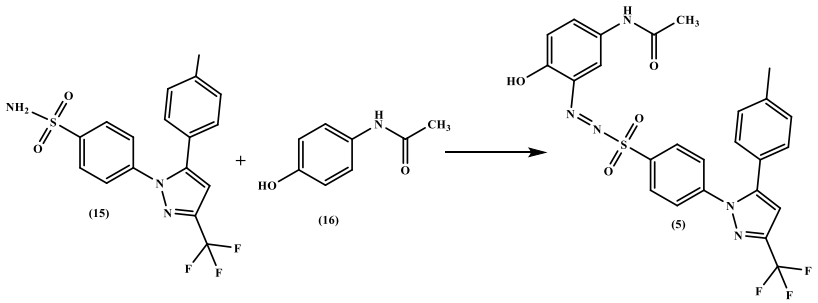


Figure (5): Synthesis of (5).

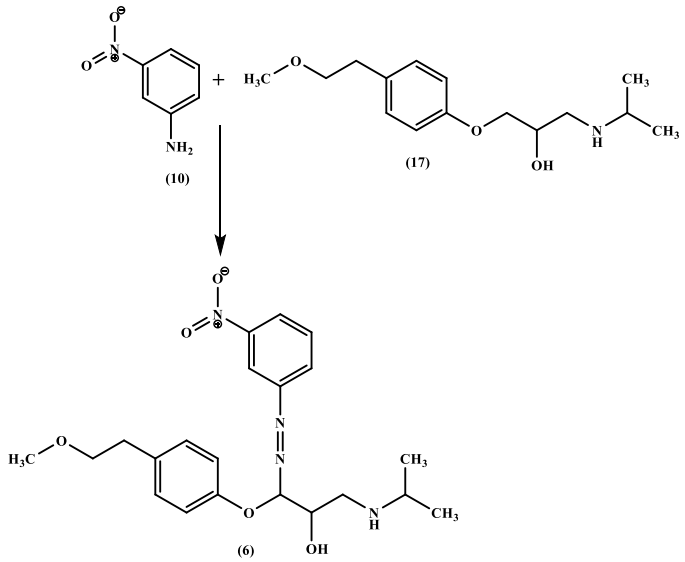
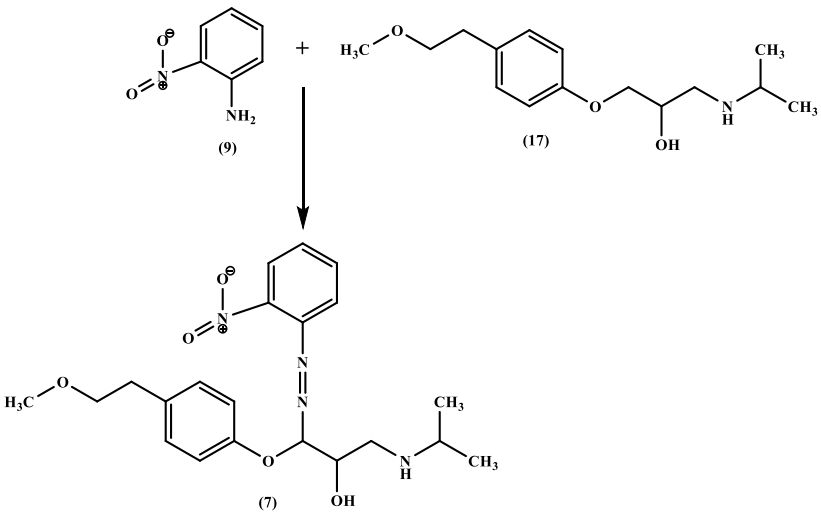


Figure (6): Synthesis of (6).



(7): Synthesis of (7).

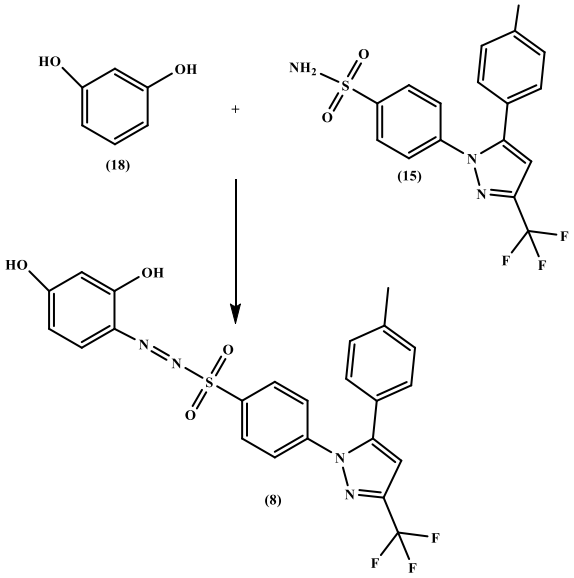


Figure (8): Synthesis of (8).

The diazo dyes, (1), (2), (3) and (4) were derived from azo dyes (*E*)-4-((2-nitrophenyl)diazenyl)benzene-1,3-diol (9)[18], (*E*)-4-((3-nitrophenyl) diazenyl)benzene-1,3-diol (10)[18] and (*E*)-4-((4-nitrophenyl)diazenyl)benzene-1,3-diol (11)[18]using a method similar to that designated by Fox[17] with optimize the stoichiometry and the conditions of the reactions. Then, the azo dyes (5), (6), (7) and (8) synthesised using same method. The azo dyes (1), (2), (3) and (4) were characterized by IR, UV-visible and mass spectrum. But, the (5), (6), (7) and (8) were characterized by IR, UV-visible and ^{1}H NMR spectrum. The UV-visible spectrums were documented at the range (250-450) nm for each synthetic azo dye. The results were showed that the maximum wave length of diazo dyes (1), (2), (3) and (4) were equal to (290, 360) nm,(290, 390) nm,(280, 370) nm and (280, 360) nm respectively as seen in the Figure (9) below.

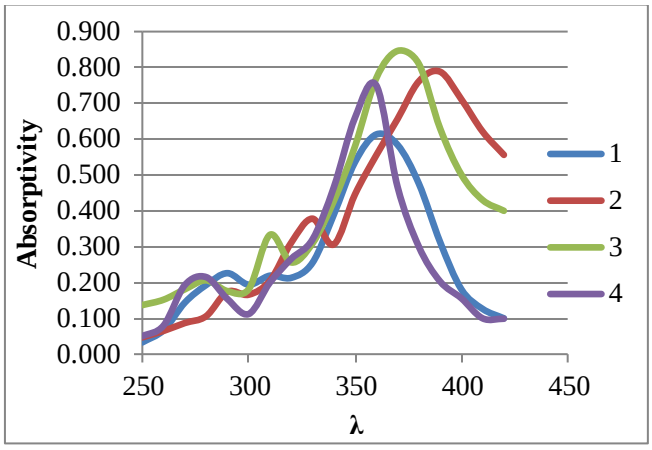


Figure (9): The UV-vis spectrum of the new diazo dyes (1-4).

The absorption spectrum of synthetic diazo dyes (1), (2), (3) and (4) were showed bands at 290 nm, 290nm, 280 nm, 280 nm, 360 nm, 390 nm, 370 nm and 360 nm related to (π -π*) and (n-π*) respectively. The results of UV-visible spectrum of (5), (6), (7) and (8) were showed that the maximum wave length were equal to (280, 350) nm, (280, 380) nm, (280, 400) nm and (270, 310) nm respectively as seen in the Figure (10) below.

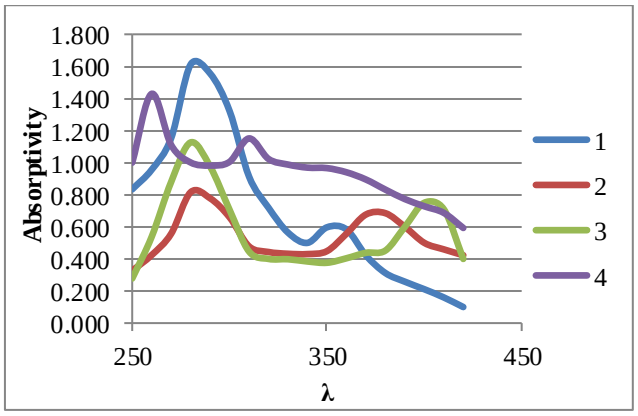


Figure (10): The UV-vis spectrum of the novel azo dyes (5-8).

The absorption spectrum of azo dyes (5), (6), (7) and (8) were showed bands at 280 nm, 280 nm, 280 nm, 270nm, 350 nm, 380 nm,400 nm and 310 nmrelated to (π -π*) and (n-π*) respectively. All these results were then compared with that obtained by ChemBio 3D Ultra - [Chem3D XML] Gaussian Interface and were seems to be identical. Add to which, the IR spectrum of the prepared diazo dyes (1), (2), (3) and (4) were showed the stretching vibration of the ν (OH) groupsin the region 3429.43cm^{-1}, 3466.08 cm^{-1}, 3464.15cm^{-1} and 3468.37cm^{-1} respectively. But, the ν (N=N) stretching vibration band was appeared in the region 1444.68 cm^{-1}, 1431.18 cm^{-1}, 1450.47 cm^{-1} and 1448.54 cm^{-1} respectively.[2,3] Other bands with this region can be considered as skeletal vibrations, the(C=C) stretching vibration of the aromatic ring shows a strong band in the region 1514.12 cm^{-1}, 1525.69 cm^{-1}, 1525.69cm^{-1} and 1521.84 cm^{-1}respectively.[2,3]And the aromatic CH bands were appeared in the region3089.96 cm^{-1}, 3088.03 cm^{-1}, 3089.96 cm^{-1} and 3107.30 cm^{-1} respectively.[3] Also, the IR spectrum of the azo dyes (5), (6), (7) and (8) were showed the stretching vibration of the ν (OH) groups in the region 3338.78 cm^{-1}, 3425.58 cm^{-1}, 3433.29 cm^{-1} and 3255.84 cm^{-1} respectively. But, the ν (N=N) stretching vibration bands were appeared in the region 1446.61 cm^{-1}, 1456.26 cm^{-1}, 1355.96 cm^{-1} and 1490.97 cm^{-1},the ν (SO_2) in the (5) and (8) were appeared in the region 1348.24 cm^{-1} and 1300.02cm^{-1}respectively,[21]other bands with this region can be considered as skeletal vibrations, the(C=C) stretching vibration of the aromatic ring shows a strong band in the region 1500.00 cm^{-1}, 1525.69cm^{-1}, 1527. 62cm^{-1} and 1490.97cm^{-1}respectively.[2,3] And the aromatic CH bands were appeared in the region3099.61 cm^{-1}, 3086.11 cm^{-1}, 2950.00 cm^{-1} and 2924.09 cm^{-1} respectively.[2,3] Thus, the peak at *m/z* 408.6 was confirmed the founding of the diazo dyes. Add to which, the ^{1}H NMR spectrum was confirmed the formation of the pharmaceutical azo dyes; (all Figures were attached in the end of the paper).

The method of Xian-guo and Ursola[13] was then applied to measure the toxity of all synthesised dyes usinghemolytic red blood cellsin vitro. The results were showed that all prepared compounds were provided non-toxic effectsand didn't show any hemolysis effect in the cells, using different concentrations of each.

The cell viability (cytotoxicity assay) is used for the diazo dyes and the pharmaceutical azo dyes screening to observe their ability in destroying cancer living cells and reduce living cells growth for human breast MDA-MB231 cancer cells. After 24h treatment with 100µM for each dye, the results were obtained as seen in Table (1) below.

Table (1): The values of cell viability after 24h of treatment with 100µM of diazo dyes

Sample	Viability% 1	Viability% 2	Viability% 3
(1)	45.5947137	70.6827309	77.6386404
(2)	45.154185	59.6385542	75.4919499
(3)	67.8414097	69.4779116	93.3810376
(4)	40.0881057	51.6064257	54.2039356
(5)	36.56388	41.56627	61.71735
(6)	51.32159	45.98394	55.27728
(7)	70.92511	72.48996	59.21288
(8)	42.51101	43.7751	53.84615
Control	100	100	100

The results of cytotoxicity were presented well action of diazo dyeson cancer cellsin

contrast with the control, especially inazo dye (5).Further, the human DNA binding of each dye was studied as seen in Figure (11) below.

Figure (11): The human DNA binding witheach azo dyeand the control (C).

The figure displays that the diazo dyes and the pharmaceutical azo dyes were effect the human DNA resulting DNA damage, which inhibits DNA transcription and replication.The human DNA concentration before and after binding with each azo dye (1-8) was measured using a NanoDrop™ spectrophotometer at A 260 nm as seen in Table (2) below. The DNA36 was the control of the diazo dyes (1-4), but the DNA62 wasthe control of the pharmaceutical azo dyes (5-8).

Table (2): The human DNA concentration before and after binding with each azo dye

Sample	concentration in g/µl
DNA 36	10
DNA62	247.5
1	5
2	7.5
3	20
4	62.5
5	145
6	177.5
7	20
8	715

The results were showed high concentrations ofnitrogen basesin contrast with the DNA control, which indicate that the binding of each synthetic azo dyewas effect the double helix of the DNA. The rises of the concentration of the nitrogen bases were showed the DNA damage. Due to, recommend these non-toxic dyes as novel anti-cancer drugs that target DNA.

Summary

The diazo dyesand the pharmaceutical azo dyes can be prepared inexpensively because the starting materials are obtainable and most of the chemistry is done at or below room temperature. Also, all the synthesised dyes gained good color and delivered non-toxic effects and they didn't show any hemolysis effect in the cells. Add to which, its ability to bind breast cancer MDA-MB231 cells and affect the cell viability%. And its ability to damage DNA, which inhibit DNA transcription and replication, Owing to recommend these dyes as novel anti-cancer drugs can damage DNA of cancer cells.

Acknowledgment

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Conflicts of interest

Authors declare that there is no conflict of interest.

تحضير اصباغ الازو الجديد كمضادات لخلايا مرض سرطان الثدي من نوع MDA-MB231 وطبيعة ارتباطها مع الحمض النووي الريبوزي منقوص الاوكسجين (DNA)

صالح جوحي عيدي، حنان مرتضى علي، علي عبد الواحد الشاوي

الخلاصة

لقد تم تحضير أصباغ الازو ثنائية الارتباط و أصباغ الازو الدوائية الآتية:

4,6-*bis*((*E*)-(3-nitrophenyl)diazenyl) benzene-1,3-diol (**1**), 4-((*E*)-(3-nitrophenyl)diazenyl)-6-((*E*)-(4-nitrophenyl)diazenyl)benzene -1,3-diol (**2**), 4-((*E*)-(2-nitrophenyl) diazenyl)-6-((*E*)-(3-nitrophenyl)diazenyl)benzene-1,3-diol (**3**), 4,6-*bis*((*E*)-(2-nitrophenyl) diazenyl)benzene-1,3-diol (**4**), (*N*-(4-hydroxy-3-(((4-(5-(*p*-tolyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl)phenyl)sulfonyl)diazenyl)phenyl)acetamide (**5**), 3-(isopropylamino)-1-(4-(2-methoxyethyl)phenoxy)-1-((3-nitrophenyl)diazenyl)propan-2-ol (**6**), 3-(isopropylamino)-1-(4-(2-methoxyethyl)phenoxy)-1-((2-nitrophenyl)diazenyl)propan-2-ol (**7**) and 4-(((4-(5-(*p*-tolyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl)phenyl)sulfonyl) diazenyl)benzene-1,3-diol (**8**) باستخدام الطريقة المستعملة في التحضير و الموصوفة من قبل فوكس. ثم شخصت أصباغ الازو ثنائية الارتباط و أصباغ الازو الدوائية المحضرة باستخدام درجة الانصهار و (مطيافية الأشعة تحت الحمراء و مطيافية الأشعة المرئية و فوق البنفسجية وطيف الكتلة) و (مطيافية الأشعة تحت الحمراء و مطيافية الأشعة المرئية و فوق البنفسجية و مطيافية الرنين النووي المغناطيسي) على التوالي. بالإضافة إلى أن جميع الأصباغ المحضرة غير سامة ولا تسبب انحلال خلايا الدم الحمراء. لقد تم أيضا القيام بفحص السمية الخلوية لكل صبغة ومراقبة قدرتها على تدمير الخلايا السرطانية لمرض سرطان الثدي من نوع MDA-MB231 بعد المعالجة لمدة24 ساعة لكل صبغة 100µM. و كانت النتائج أن هذه الأصباغ تقلل من تواجد الخلايا السرطانية بشكل واضح بالمقارنة بالخلايا المرجع. كما تم دراسة ارتباط الأصباغ المحضرة مع الحمض النووي منقوص الأوكسجين ال DNA البشري و كانت النتائج أن هذه الأصباغ تحطم ال DNA وتثبط استنساخه و تكراره. كما أكدت نتائج مقياس الطيف الضوئيNanoDrop™ spectrophotometer ارتفاع تركيز القواعد النيتروجينية مما أكد تحطم الDNA . لذلك نوصي باستخدام هذه الأصباغ كمضادات غير سامة لمرض سرطان الثدي من نوع MDA-MB231 و يمكنها تحطيم ال DNA لتلك الخلايا.

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