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Synthesis and Characterization of Some New Azo Dyes Derivatives Via Chalcone and Study Some of Their Biological Activity

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Abstract:

This work includes synthesis of new six membered heterocyclic rings with effective amino group using the reaction of benzylideneacetophenone (chalcone) (1) with thiourea or urea in alcoholic basic medium to form: 1,3-thiazin-2-amine (2), and 1,3-oxazin-2-amine (8) respectively.

The diazotization reaction was carried out with sodium nitrite in presence of hydrochloric acid to form diazonium salts which suffered coupling reaction with naphthols and phenols in the presence of sodium hydroxide to form colored azo dyes (4-7, and 10-13).

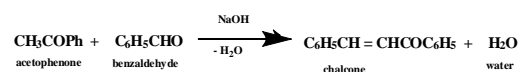
o-methylation reaction of compounds (7) and (10) yielded : 1,3-thiazin -2-yl-diazenyl (14), and 1,3-oxazin-2-yl-diazenyl (15) respectively.

The new compounds were characterized using various physical techniques like: UV-Vis., FT-IR, and ¹HNMR spectra. Some new compounds were tested against bacteria.

Key words: Heterocyclic, Azo dyes, Benzylideneacetophenone, Thiazine, Oxazine.

Introduction:

Chalcone is an α,β -unsaturated ketone, produced from aldol condensation reaction of acetophenone with benzaldehyde or substituted benzaldehyde in presence of sodium hydroxide as a catalyst as shown below [1-4]:



Its general formula $\text{C}_6\text{H}_5\text{CH}=\text{CHCOPh}$ has two isomers cis and trans, trans isomer is more common[1,3]. It has several names such as : benzylideneacetophenone,

benzalacetone, and methyl styryl ketone [5]., The name "Chalcone" was given by Kostanecki and Tambor; Chalcones are well known intermediates for synthesizing various heterocyclic compounds [3,4].

Chalcones can be isolated from several plants, and are precursors of flavones compounds, they are similar to enamines, they contain a double bond and prepared from intervention condensation reaction with urea or thiourea and other organic nitrogen compounds to form heterocyclic compounds[3], the reaction should carried out with strong basic conditions and low temperatures to

yield compounds used in pharmaceutical and an industrial purposes[1,6].

When a primary aromatic amine is treated with nitrous acid at low temperature, it will converted to a diazonium salt, the simplest form is benzenediazonium chloride [7,8]. Coupling reaction of azo dye is an organic electrophilic substitution reaction between a diazonium salts and other aromatic compound [8], the products will absorb longer wavelengths of light especially in the visible region compared with the reactants because of the conjugation ,therefore, aromatic azo dyes tend to be brightly colored due to the extended conjugated system[8,9]. Also products of azo coupling reaction used to form pharmaceutical drugs such as sulfa drugs[10,11].

The different application fields of synthetic azo compounds are widely such as foods, medicines, cosmetics, paints, shipbuilding, automobile, plastics,industry,cable manufacture, etc. [12,13].However, the traditional application field of the synthetic azo dyes still remains the textile industry, and the finishing of fibrous materials in order to impart simultaneously with coloration[6,13]. Using gold (Au) nanoparticles supported on TiO₂ as a catalyst in the the aerobic oxidation of aromatic anilines to aromatic azo compounds, also using Au as a hydrogenation catalyst on TiO₂ making it possible to prepare azo compounds directly from nitro aromatics through a two-step (hydrogenation followed by aerobic oxidation), one-pot, and one-catalyst reaction. In addition, the catalytic process is efficient for the synthesis of symmetric and a range of asymmetric aromatic azo compounds from the mixtures of two anilines substituted with electron-donor and electron-acceptor constituents [14].

The aim of this work is to prepare and characterize new series of azo compounds starting from α , β -unsaturated ketone which it were expected to have a biological activity.

Material and Methods:

All chemicals were used through this work purchased from Fluka, Merck Companies and were used without further purifications.

Melting points were recorded using a measuring device melting point type: melting point (SMP 30) Stuart and were uncorrected. Thin layer chromatography (T.L.C.) was carried out using Fertigfolien precoated sheet type: Polygram silica- gel as stationary phase, ethyl acetate as eluent, and the spots were developed with iodine vapor. U.V-Vis. spectra were recorded with spectrophotometer type: SHIMADZU UV spectrophotometer - 1800 using DMSO as a solvent. Infrared spectra were recorded using Fourier transform infrared SHIMADZU(8300) (FT.IR) infrared spectrophotometer by KBr disc. ¹HNMR spectra were recorded on Fourier Transform Varian spectrophotometer, operating at 300 MHz with tetramethylsilane as internal standard, measurements was made at Chemistry Department, AL-AL-BAYT University, Jordan. The biological activity was performed by Biology Department, College of Science, Baghdad University.

Synthesis of 4,6-diphenyl-2H-1,3-thiazin-2-amine(2)[6]

Benzylideneacetophenone (0.01mol, 2.08gm) (1) was dissolved in alcoholic sodium hydroxide (20mL, 40%) then thiourea (0.01mol, 0.76 gm) was added to it. The reaction was stirred in an ice bath at (5-10) °C for two hours , then added to the appropriate amount of ice water , the mixture was stirred for (1) hour. The mixture was kept in

refrigerator for (24) hours, then filtered. A yellow solid material was collected, m.p (113-115) °C and (2.15gm, 81% yield).

Synthesis of *E*-4-((4,6-diphenyl-2*H*-1,3-thiazin-2-yl)diazenyl)naphthalene-1-ol(4) [15,16]

Compound (2) (0.065mol,1.729gm) was added to a solution of hydrochloric acid (10 mL HCl +10 mL water) and cooled in ice-salt bath at (0-5) °C with stirring . To this solution a cold sodium nitrite solution (0.448gm in 3mL) water was added slowly with stirring at (5) °C for (1) hour to form diazonium salt(3).,1-Naphthol (0.936gm) was dissolved in diluted sodium hydroxide solution (10%) and cooled in ice-salt bath at (0-5) °C. To this solution diazonium salt (3) was added with stirring at (5) °C. The colored solid crystals were filtered, washed with distilled water, dried ,to obtain compound (4), m.p (161-163) °C and (2.73gm, 79% yield).

Compounds (5,6 and 7) were prepared by same procedure.

Synthesis of 4,6-diphenyl-2*H*-1,3-oxazin-2-amine(8)

Chalcone (0.01mol, 2.08gm) (1) was dissolved in alcoholic sodium hydroxide (20 mL, 40%) then urea (0.01mol, 0.6gm.) was added to it. The reaction was stirred in an ice bath at (5-10) °C for two hours, then added to the appropriate amount of ice water, the mixture was stirred for (1) hour. The mixture was kept in refrigerator for (24) hours, then filtered. A yellow solid was collected, m.p (83-85) °C and (2. 5gm, 77% yield).

Synthesis of *E*-4-(4,6-diphenyl-2*H*-1,3-oxazin-2-yl) diazenyl)naphthalene -1-ol(10) [15,16]

Compound (8) (0.065mol,1.625gm) was added to a solution of hydrochloric acid (HCl 10mL + water10 mL) and cooled in ice-salt bath at (0-5) °C with stirring . To this solution a cold sodium nitrite solution (0.448 gm) in water (3mL) was added slowly with stirring at (5) °C for (1) hour to form diazonium salt (9)., 2-Naphthol (0.936gm) was dissolved in diluted sodium hydroxide solution (10%) and cooled in ice-salt bath at (0-5) °C. To this solution diazonium salt (9) was added with stirring at (5) °C. The colored solid crystals were filtered, washed with distilled water, dried , to obtain compound (10), m.p (153-155) °C and (2.12gm, 81% yield). Compounds (11,12 and 13) were prepared by same procedure.

Synthesis of *E*-2-((4-methoxy-3,5-dimethylphenyl)diazenyl)-4,6-diphenyl-2*H*-1,3-thiazin(14) and *E*-2-((4-methoxynaphthalene-1-yl)diazenyl)-4,6-diphenyl-2*H*-1,3-oxazin(15)[15]

Compound (7) (0.001mol,0.399gm) was dissolved in diluted sodium hydroxide solution (0.1mL , 10%) and cooled in an ice-bath at (10-15) °C with stirring. To this solution dimethylsulfate (0.001mol, 0.126 gm, 0.1 mL) was added drop wise ; Warm the mixture in water bath at (70-80) °C for (2) hours. The brown solid crystals were filtered, washed with water, dried to obtain compound (14), m.p (203-206) °C and (0. 85gm, 61% yield).

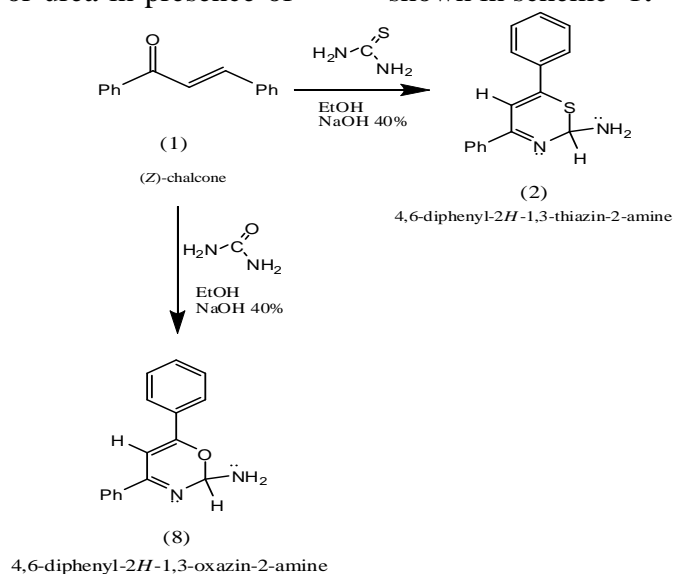
Compound (15) was prepared by same procedure, m.p (167-170) °C and (0.97gm, 74% yield).

Results and Discussion:

The compounds (2) and (8) were prepared using the reaction between

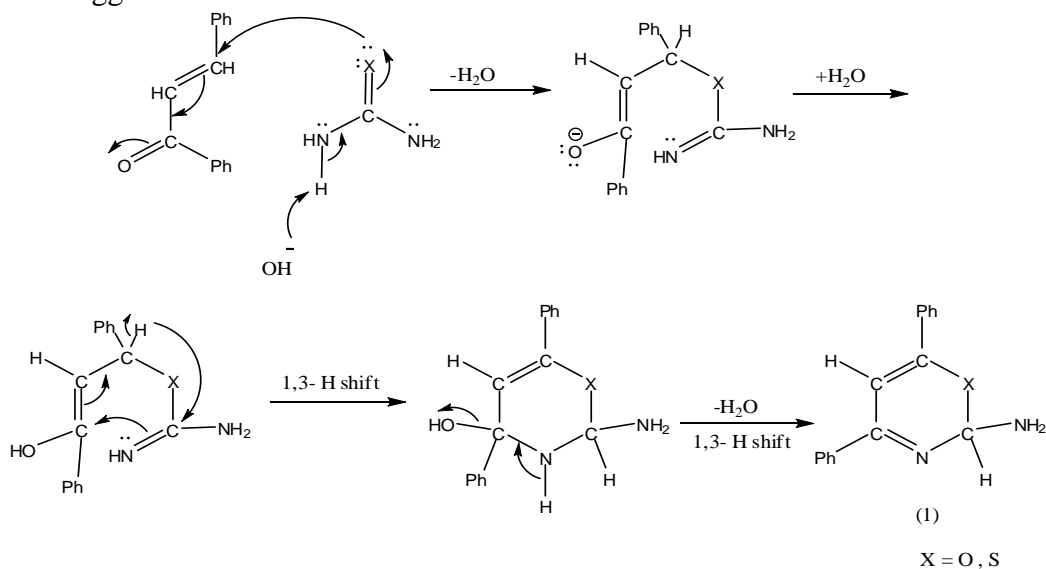
benzylideneacetophenone (chalcone) (1) and thiourea or urea in presence of

sodium hydroxide solution (40%) as shown in scheme -1:



Scheme- 1

The suggested mechanism is :



FT-IR spectrum of compound (2), disappeared the bands at $(1664) \text{ cm}^{-1}$ due to stretching vibration of carbonyl group ($\text{C}=\text{O}$), and $(1245) \text{ cm}^{-1}$ due to stretching vibration of thion group ($\text{C}=\text{S}$).

The moderate bands at $(1350) \text{ cm}^{-1}$ and $(692) \text{ cm}^{-1}$ are attributed to stretching vibrations of ($\text{C}-\text{N}$) and ($\text{C}-\text{S}$) respectively; The bands at $(3011) \text{ cm}^{-1}$, $(2949) \text{ cm}^{-1}$, and $(2920) \text{ cm}^{-1}$ are attributed to stretching

vibrations of ($\text{C}-\text{H}$) alkene, aromatic, and aliphatic respectively.

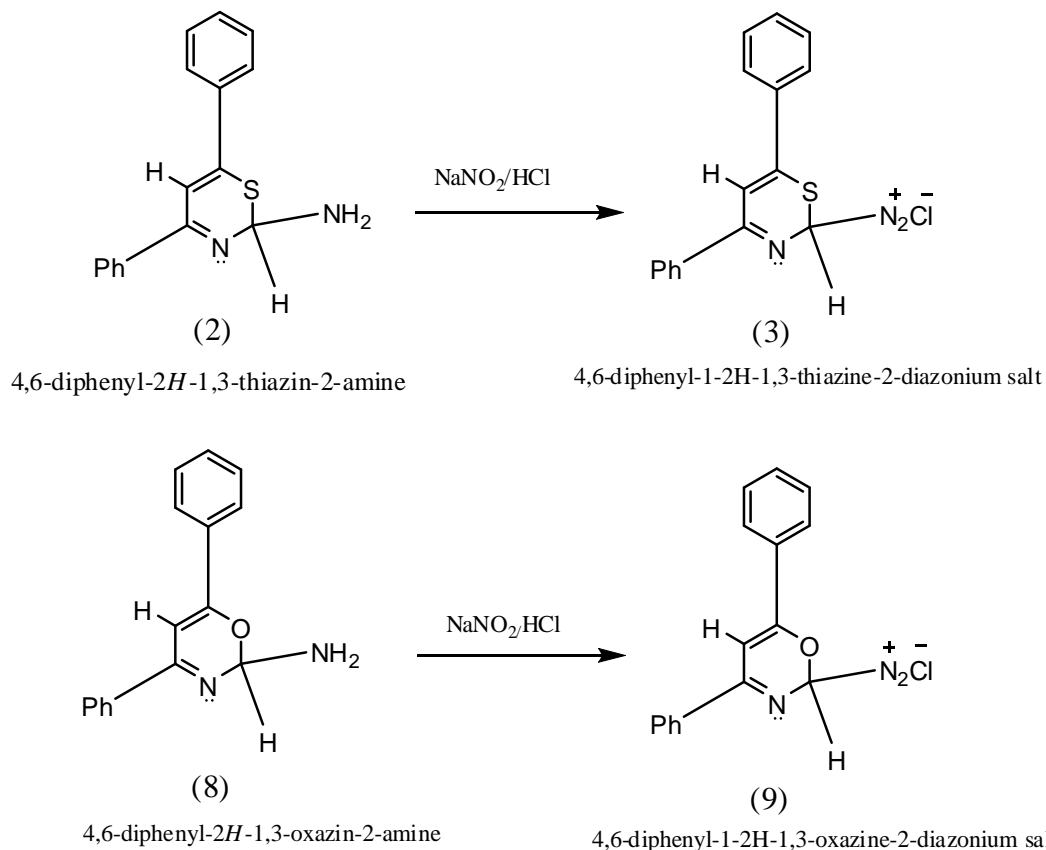
FT-IR spectrum of compound (8), displays the bands at $(1664.57) \text{ cm}^{-1}$, and $(1676) \text{ cm}^{-1}$ due to stretching frequency of carbonyl group ($\text{C}=\text{O}$) of starting materials. The bands at $(1319) \text{ cm}^{-1}$ and $(1292) \text{ cm}^{-1}$ are attributed to ($\text{C}-\text{N}$) and ($\text{C}-\text{O}$) stretching vibration respectively. The bands at $(3192) \text{ cm}^{-1}$, $(3084) \text{ cm}^{-1}$, and $(2945) \text{ cm}^{-1}$ are attributed to stretching vibrations of

(C-H) alkene, aromatic, and aliphatic respectively (table-1)

Table (1): FT.IR spectral data of compound [2]and [8].

Comp. no.	ν (NH ₂)	ν (C=N)	ν (C=C) alkene	ν (C-N)	ν (C-S)	ν (C-O)
(2)	Asym. 3441 Sym.3213	1626	1574	1350	692	-
(8)	Asym.3419 Sym. 3316	1618	1568	1310	--	1292

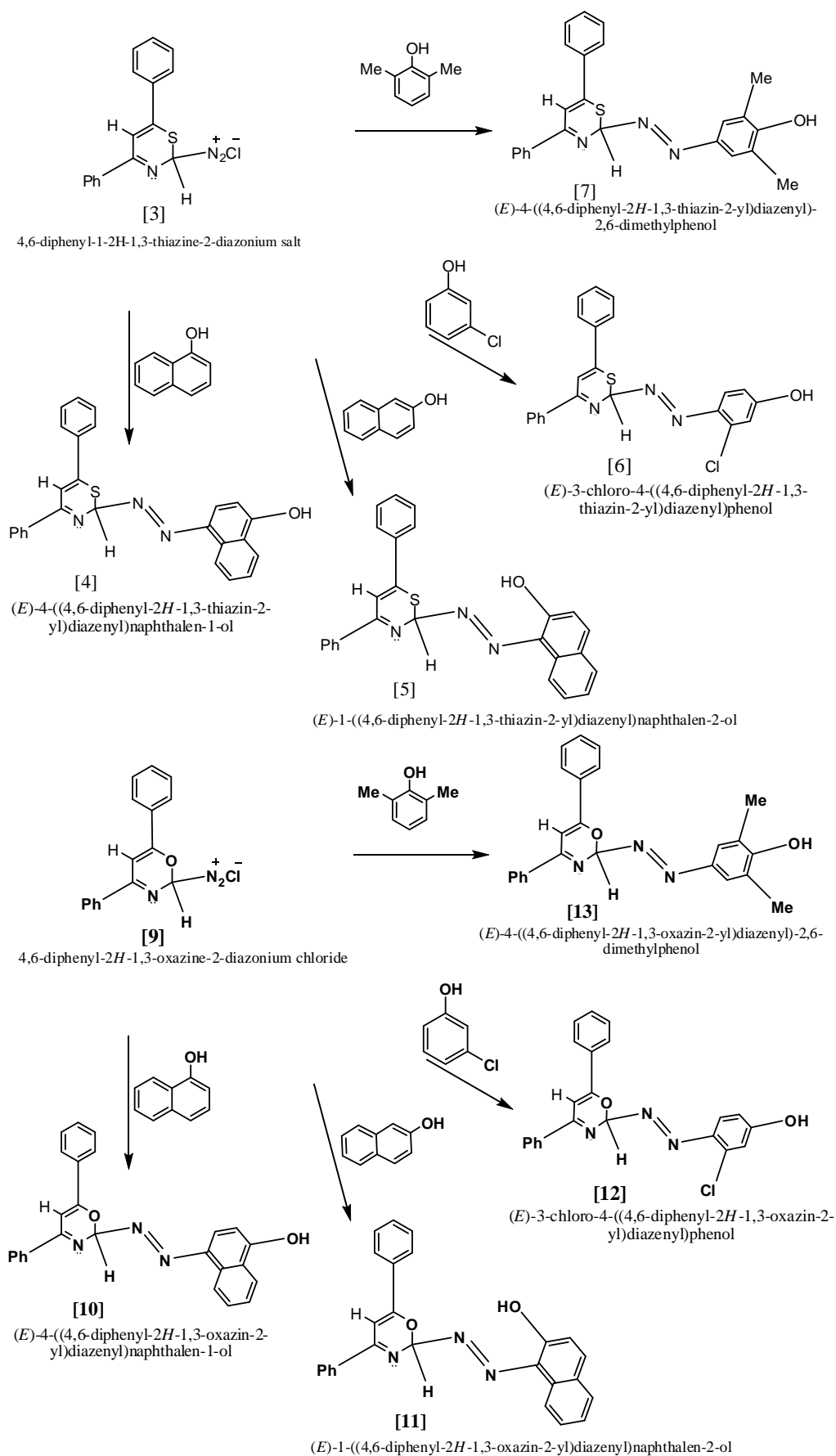
Diazotization of compounds (2) and (8) were carried out by reaction with sodium nitrite in presence of hydrochloric acid as shown in scheme-2 [15-18]:



Scheme- 2

colored compounds as shown in scheme-3 [15-18] :

The coupling reactions were carried out by interactions of diazonium salts with naphthols and phenols to yield



Scheme- 3

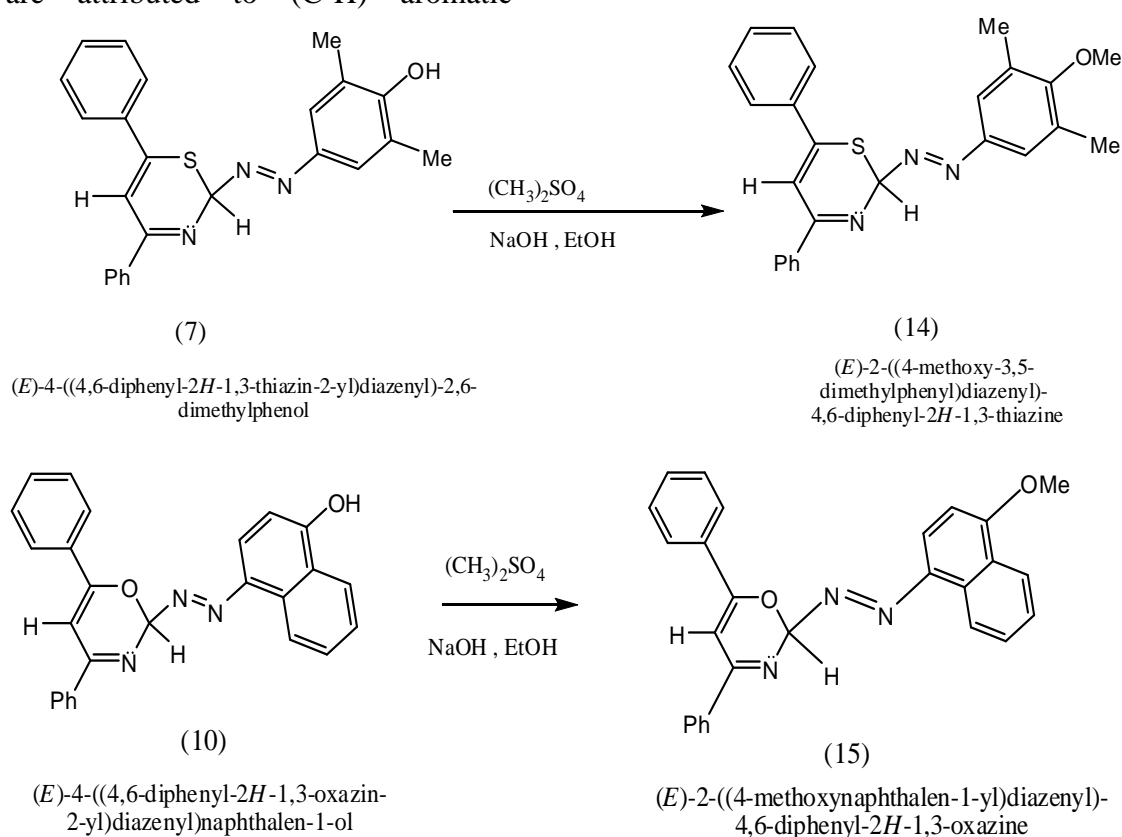
FT-IR spectra of dyes compounds (4),(5),(6),(7),(10),(11),(12) and (13) showed disappearance of the bands at $(3400) \text{ cm}^{-1}$ and $(3300) \text{ cm}^{-1}$ due to amino group (NH_2) for compounds (2 and 8), and appearance of bands at $(1593-1522) \text{ cm}^{-1}$ due to (trans $\text{N}=\text{N}$), and at $(3465-3329) \text{ cm}^{-1}$ due to (phenolic OH) ; This supports the incidence of coupling reaction successfully.

The bands at $(3062) \text{ cm}^{-1}$, $(3051) \text{ cm}^{-1}$, $(3011) \text{ cm}^{-1}$, $(3020) \text{ cm}^{-1}$, $(3045) \text{ cm}^{-1}$, $(3047) \text{ cm}^{-1}$, $(3086) \text{ cm}^{-1}$, and $(3064) \text{ cm}^{-1}$ are attributed to (C-H) aromatic

stretching ; The bands at $(980) \text{ cm}^{-1}$ and $(994) \text{ cm}^{-1}$ attributed to (C-Cl) aromatic stretching respectively for compounds (6 and 12).

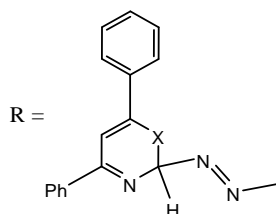
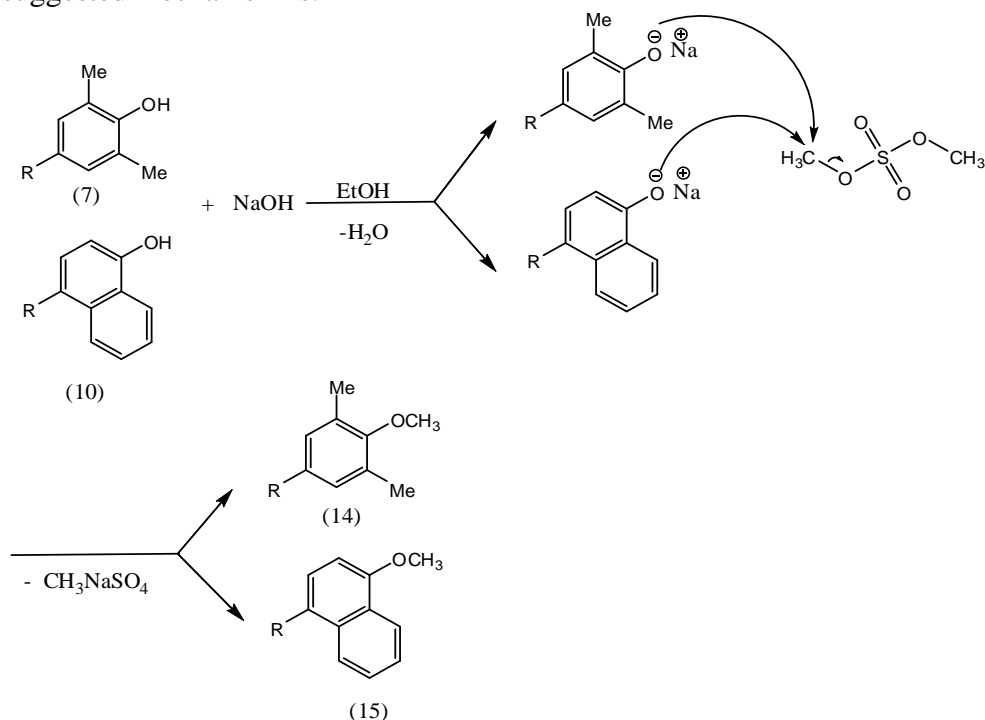
FT-IR spectrum of compound (5), showed absorption band at lower frequency due to intramolecular hydrogen bonding between acidic phenol proton and electronic pair of azo group nitrogen [19] (table-2).

Compounds (7) and (10) can be methylated readily with dimethyl sulfate (DMS) forming ethers (14) and (15) as shown in scheme-4 [15].



Scheme- 4

The suggested mechanism is:



X = S, O

Table (2) :FT-IR spectral data of compounds (4), (5), (6), (7), (10), (11), (12) and (13).

Co mp. no.	ν (O-H) phenolic	ν (C=C) aromatic	ν (C=C) alkene	ν (C=N)	ν (N=N) trans azo	ν (C-N)	ν (C-S)	ν (C-O)
(4)	3449	1649 & 1449	1662	1670	1575	977	689	---
(5)	3465	1630 & 1467	1683	1693	1575	983	684	---
(6)	3464	1608 & 1458	1699	1720	1573	962	680	---
(7)	3404	1576 & 1456	1621	1616	1575	981	690	---
(10)	3386	1443 & 1437	1618	1617	1593	1279	---	1225
(11)	3360	1489 & 1383	1608	1672	1530	1298	---	1169
(12)	3329	1499 & 1437	1614	1672	1589	1288	---	1217
(13)	3387	1482 & 1420	1624	1676	1522	1300	---	1213

FT-IR spectrum of compound (14), showed disappearance of (OH) band at (3404) cm^{-1} , and appearance the band at (1193) cm^{-1} attributed to (C-O) ether group. The bands at (3045.6) cm^{-1} , (2972) cm^{-1} , and (2987.7) cm^{-1} are attributed to (C-H) aromatic stretching, (C-H) aliphatic stretching and (C-H) alkene stretching respectively (table- 3).

FT-IR spectrum of compound (15), showed disappearance of (OH) band at (3386) cm^{-1} , and displays the band at (1170) cm^{-1} attributed to (C-O) ether group. The bands at (3050) cm^{-1} , (2993, 2931) cm^{-1} , and (3012) cm^{-1} are attributed to (C-H) aromatic stretching, (C-H) aliphatic stretching, and (C-H) alkene stretching respectively (table -3).

Table (3): FT.IR spectral data of compounds [14] and [15].

Comp. no.	ν (C=C) aromatic	ν (C=C) alkenic	ν (C=N)	ν (N=N) trans azo	ν (C-N)	ν (C-O)	ν (C-S)
(14)	1562 & 1485	1584	1612	1583	1219	1193 ether	696
(15)	1425 & 1387	1603	1713	1584	1203	1170 ether	-

UV. - Vis. spectra of the prepared compounds were showed high intense absorption peaks at the range (310-220) nm which assigned to overlap of ($\pi - \pi^*$) and ($n - \pi^*$) transitions [18,19].

^1H NMR spectrum of compound (2), showed a singlet signal at $\delta = 2.503\text{ppm}$ attributed to a proton bonded to (C5) in thiazine ring, and singlet signal at $\delta = 3.348\text{ppm}$ due to a proton bonded to (C2) of thiazine ring; while a multiplate signal at $\delta = 7.191-8.433\text{ppm}$ due to interference of amino and phenyl protons.

^1H NMR spectrum of compound (4), showed a singlet signal at $\delta = 2.303\text{ppm}$ due to proton on (C5) of thiazine ring and another singlet signal at $\delta = 3.230\text{ppm}$ due to proton on (C2) of thiazine ring; A multiplate signal at $\delta = 7.452 - 8.167\text{ppm}$ due to naphtholic proton interference with phenyl protons. ^1H NMR spectrum of compound (8), showed a singlet signal at $\delta = 2.501\text{ppm}$ attributed to a proton bonded to (C5) in oxazine ring, and singlet signal at $\delta = 3.341\text{ppm}$ due to a proton bonded to (C2) of oxazine ring; while a multiplate signal at $\delta = 7.00 - 7.88\text{ppm}$ due to interference of amino and phenyl protons. ^1H NMR spectrum of compound (12), showed a singlet signal at $\delta = 2.156\text{ppm}$ due to proton on (C5) of oxazine ring and another δ singlet signal at $\delta = 3.301\text{ppm}$ due

to proton on (C2) of oxazine ring; A multiplate signal at $\delta = 7.436-9.118\text{ppm}$ due to interference of phenolic proton and phenyl protons.

Table (5) showed the physical properties of the prepared compounds.

Table (5): Physical constants of the prepared compounds.

Comp. no.	M.p. $^{\circ}\text{C}$	Yield %	Color	Recryst. solvent
2	113 - 115	75	Yellow	EtOH
4	161 - 163	70	Yellow	EtOH
5	151 - 154	80	Yellow	EtOH
6	175 - 176	70	Deep brown	EtOH
7	191 - 193	65	Reddish brown	EtOH
8	83 - 85	73	Deep yellow	EtOH
10	153 - 155	60	Red - orange	EtOH
11	147 - 149	61	Red - brown	EtOH
12	156 - 157	67	Deep brown	EtOH
13	179 - 181	73	Brown	EtOH
14	203 - 205	60	Brown	EtOH
15	167 - 170	65	Light brown	EtOH

Antibacterial activity

In this work, the antibacterial test was performed according to the disc diffusion method. Compounds (4, 12 and 14) were assayed for their antimicrobial activity *in vitro* against Gram-negative bacteria (*Escherichia Coli*) and Gram-positive bacteria (*Staphylococcus aureus*). Prepared agar and Petri dishes were sterilized by autoclaving for 15min. at 121C° . The agar plates were surface inoculated uniformly from the broth culture of the tested microorganisms. In the solidified medium suitably spaced apart holes were made all 6 mm in diameter. These holes were filled with 0.1 ml of the prepared compounds (20mg of the compound dissolved in 1mL of DMSO solvent), DMSO was used as a solvent. These plates were incubated at 37C° for 24hr for

bacteria. The inhibition zones caused by the prepared compounds were examined. The results of the preliminary screening tests are listed in (table- 6).

Table (6): Results of antibacterial activity of the tested compounds.

Comp. no.	Escherichia Coli	Klebsiella Pneumonia
(4)	+	-
(12)	-	-
(14)	++	-

- = No inhibition = inactive., + = (5-10) mm = slightly active., ++ = (11-20) mm = moderately active

References:

- [1] Palleros D.R. 2008. Synthesis of Dihydroxylated Chalcone Derivatives with Diverse Substitution Patterns and Their Radical Scavenging Ability toward DPPH Free Radicals. *J. Chem. Educ.*, 81:1345-1351.
- [2] Faqrodoin, S., Rahaman, A., and Moinuddin, M. 2012. Chalcone: A Versatile Molecule. *International journal of life science and pharma research*. 2(1):186-193.
- [3] Venkatesan, P., and Sumathi, S. 2010. Multi component Reactions for the Synthesis of Heterocycles. *J. Heterocyclic Chem.*, 47(81):489-497.
- [4] Hasan, A., and Rasheed, A. 2007. Synthesis and Biological Evaluation of Chalcone Derivatives Linked Triazoles. *Asian J. Chem.*, 19(2) : 937-943.
- [5] Sundararajan, R., Kamalakkannan, D., John, S., Joseph, K., and Subramanian. G. 2013.
- [6] Solvent-free synthesis, spectral correlations and antimicrobial activities of some 2-, 3-, 4-trichlorophenyl chalcones. *QScience Connect*. August., 67(7):617-623
- [7] Seyed Ali Khani, M., and Naimi, M.R. 2008. Steroid Dimers: Chemistry and Applications in Drug Design and Delivery. 12th International Electronic Conference on Synthetic Organic Chemistry (ECSOC-12), Spain.
- [8] Hermenegildo, G. 1978. Chemistry of Diazonium and Diazo groups, Part 1, 2nd ed., Wiley - Blakweii.
- [9] Klaus, H., Peter, M., Wolfgang, R., Rederich, R., and Aloys E. 2005. Azo Dyes, in Ullmann's Encyclopedia of Industrial Chemistry, Wiley- VCH, Weinheim. Norman, R.H., and Rose, C. 1966. Organic Chemistry, 1st ed. New York:598-599.
- [10] Hartwell, J.L., Luis, F., and Fieser, F. 1963. Synthesis of chalcone analogues with increased anti-leishmanial activity. *Org. Synth. Coll.* 2:145-149.
- [11] Clarck, H.T., and Kinner, W.R. 1966. Synthesis, Characterization and Computational Study of Some New Heterocyclic Derived from 1-(biphenyl-4-yl)-3-(furan-2-yl)prop-2-en-1-one. *Org. Synth. Coll.* 1(1):374-378.
- [12] Rui zhao, Chunyan, T., Yonghua, X., Chunmei, G., and Yuyang, J. 2011. One step synthesis of azo compounds from nitroaromatics and anilines. *Tetrahedron Letters*. 52(29):3805-3809.
- [13] Simu, G., Dragomirescu, A., Andoni M., and Bals, G. 2010. Azo Compounds with Antimicrobial Activity; 14th International Electronic Conference on Synthetic Organic Compounds (ECSOC14) November :1-30.
- [14] Abdessamad, G., Avelino, C., and Hermenegildo, G. 2010. Preparation of symmetric and asymmetric aromatic azo compounds from aromatic amines or nitro compounds using supported gold catalysts. *Nature Protocols*. 11:429-438.
- [15] Arthur Vogel I.A. 1973. Practical Organic Chemistry., 3rd.ed. New York :219-221.
- [16] William, K. 1967. practical Organic Chemistry; 1st.ed. John Wiley :24-25 .
- [17] Cristini, C., Melone, F., and Setle, M. 2011. A Linear Oligomer Biomacro molecules; 2nd.ed., New York :392-393.
- [18] Dong-mee, S., and Kyung-hoon, J. 2002. photochemistry of chalcone

and the application of chalcone - derivatives in photo- alignment layer of liquid crystal display; Opt.Mat., 21:667-671.

[19] Shriner, Ralph, L. 1980. The Systematic Identification Of Organic Compounds; 6th.ed.,New York: 123.

تحضير و تشخيص بعض مشتقات جديدة لأصباغ الأزو من جالكون و دراسة بعض الفعالية البيولوجية لهم

ميسون طارق توفيق

هلال مسعود عبدالله

قسم الكيمياء ، كلية التربية للعلوم الصرفة - ابن الهيثم ، جامعة بغداد

الخلاصة:

تضمن البحث تحضير حلقات جديدة غير متجانسة سداسية معوضة بمجموعة الأمينو الفعالة وذلك عن طريق تفاعل بنزليدين أسيتوفينون (جالكون) (1) مع الثايويوريا أو اليوريا في وسط قاعدي كحولي و تكوين : 3,1- ثيازين-2-أمين (2) و 3,1- أوكسازين-2-أمين (8) على التوالي، ثم تحضير ملح الديازونيوم بمفاعلة (2) و (8) مع نترت الصوديوم بوجود حامض الهيدروكلوريك لتكوين المركبات (3) و (9) التي تعاني تفاعل ازدواج مع الفينولات و النفثولات بوجود القاعدة لتكوين الأصباغ الملونة (4-7 و 10-13). ان تفاعل مثيلة اوكسجين المركب (7) و (10) أعطى الايثر : 3,1- ثيازين - 2 - يل - ثنائي أزينيل (14) و الايثر: 3,1 - اوكسازين - 2 - يل - ثنائي أزينيل (15) على التوالي .

شخصت جميع المركبات الجديدة المحضرة بمطيافية الأشعة فوق البنفسجية و الأشعة تحت الحمراء و الرنين النووي المغناطيسي. أختبرت بعض هذه المركبات ضد أنواع من البكتريا.

الكلمات المفتاحية : مركبات حلقة غير متجانسة ، صبغات الأزو ، بنزليدين أسيتوفينون ، ثيازين ، أوكسازين.