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## Synthesizing, Characterizing and Studying the Biological Activity of Some New Schiff-Bases Derivatives Containing the Monosaccharide Moiety

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### Abstract:

A new series of  $\alpha$ -D-glucose as Schiff bases derivatives is synthesized and characterized with studying their bioactivity. Hydroxyl groups at C (1,2&5,6) sugar moiety are converted into acetal form through a reaction with dry acetone using phosphoric acid and anhydrous zinc chloride as catalysts producing 1,2:5,6-di-O-isopropyleidene  $\alpha$ -D-glucopyranose(I). The five membered ring acetal of C(5,6) is hydrolyzed with acetic acid (65%) and a reaction of the new product with sodium periodate is carried on to get an aldehyde moiety which is used to produce a new series of Schiff bases through reacting with different amino compounds such as 4-amino antipyrine .

The suggested chemical structures of the prepared compounds are confirmed by using UV., FT-IR and <sup>1</sup>H-NMR spectra .Most of the prepared compounds show antibacterial activity.

**Key words:** Synthesis, Biological Activity, Schiff Bases.

### Introduction:

Many sugar analogs containing nitrogen [1] sulfur [2(a,b,c)] or phosphorus [3] as a ring heteroatom have been prepared because of the wide interest in their chemical and biochemical properties. Heteroatom-in-the-ring sugar analogs of 2-amino- and 2-acetamido-2- deoxyhexo pyranoses, which widely occur as a component of many natural products, have also attracted considerable interest [4].

Acylated D-glucopyranose and D-glucopyranose derivatives are prepared and another study of *in vitro*

antimicrobial activities of furanose monosaccharides plus pyranose monosaccharide acylates has been done as a comparative study. While the (SAR) structure activity relationship study show that the acyl derivatives in six-membered pyranose form are more effective by antimicrobial functionality than that of the corresponding acyl derivatives in five-membered furanose form [5].

Schiff bases are widely used as useful organic compounds. They are used "as intermediates in organic synthesis, dyes,

pigments, polymer stabilizers, and catalysts" [6,7].

Glucosamine "Schiff bases", although known since 1922, have received relatively less attention in the literature. For the compounds that consist of the Schiff's base and glucose sub-units a possibility of tautomeric and anomeric equilibria has to be taken into consideration [8]. The study of some glucosamine Schiff bases structures by means of an initio calculations and the anomeric and tautomeric equilibria in a DMSO solution by spectroscopic methods is reported [9].

Schiff bases or imines are easily generated by condensate carbonyl groups and primary amines. In carbohydrate chemistry, a large number of imines have been reported, both by reaction of sugar aldehydes with amines and by the reaction of aminosugars with aldehydes [10-13]. Helmoz R. reports the synthesis of a series of D-glucosamine derivatives and evaluated their antimicrobial activity. Some of the investigated compounds have a significant antimicrobial activity against positive and negative germ bacterial strains as the fungal strains, so the sugar moiety is necessary due to final results, to increase the biological activity of these compounds [14].

The aim of this work is to synthesize and characterize a new series of Schiff bases and investigate their bioactivity.

### Materials and Methods:

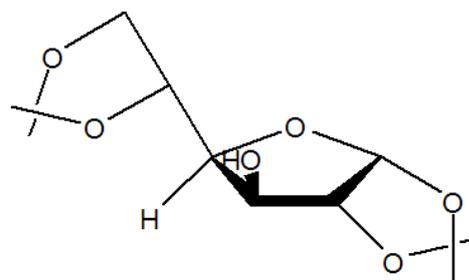
All chemicals in this work are from BDH., Fluka, Merk and Aldrich Chemicals Co. and used as received.

### Instrumentations:

Melting point is evaluated by (Stuart /Melting Point /smp30) apparatus and is uncorrected. FT- IR spectra are recorded by using KBR disc on (a Shimadzu FT-IR 8400S) Fourier Transform Infrared Spectro-photometer. UV. spectra are recorded by using a Shimadzu (UV.-

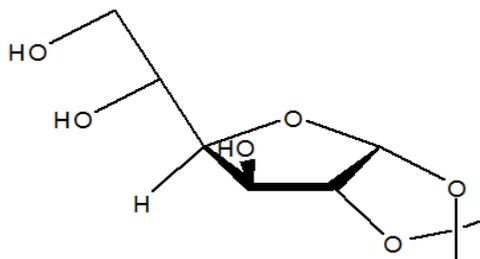
160A) UV-Vis recording Spectrophotometer. C.H.N. analysis is performed via (Euro vector EA 3000A Elemental Analyzer) in AL-al-Bayt University- Jordan. The  $^1\text{H}$ NMR spectra (DMSO) are obtained on (a Bruker - 300 MHZ Ultra Shield) with (TMS) as an internal standard in University of AL-al-Bayt- Jordan. Thin Layer Chromatography (TLC) is carried out, and the plates are developed with iodine vapor. The results of biological activity are performed in College of Science – University of Baghdad.

### "Synthesis of 1,2:5,6-Di-O-Isopropylidene - $\alpha$ -D-Glucopyranose" (I) [15]:-



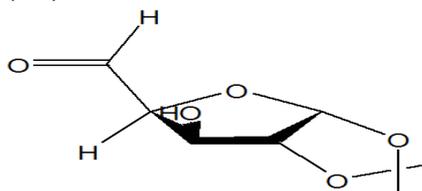
To an efficiently stirred suspension of anhydrous glucose (30gm ; 0.166 mol.) in acetone (200mL) anhydrous pulverized zinc chloride (24gm ; 0.182 mol.) is added , followed by phosphoric acid (85%;0.8 mL). This mixture is stirred at R.T. for 30 hrs., an dissolved glucose (12.4gm) is collected and washed with acetone . The washings & filtrate are cooled , resulting alkaline with a solution of sodium hydroxide ( 17 gm, in 17mL; H<sub>2</sub>O) , the inorganic material is removed by filtration ; colorless filtrate is concentrated ; the residue diluted with (50 mL; H<sub>2</sub>O ).The organic layer is extracted by (3x50 mL ;CHCl<sub>3</sub>) and dried under reduced pressure to give a white precipitate of crude di-isopropylidene glucose .The product is recrystallized from chloroform : n-hexane (1:10) (m. p.=104 -106<sup>o</sup> C ; yield = 73%),(R<sub>f</sub> =0.64 ; benzene : methanol ; 8:2).

### "Synthesis of 1,2-O-Isopropylidene- $\alpha$ -D-Glucofuranose" (II) :-



Compound (I) (20g,0.09mole) is dissolved in a mixture of acetic acid (65%,50mL) and methanol absolute (15mL),and stirred at room temperature for 72 hrs. [16]. The resulting solution is mixed with benzene (50mL)and then evaporated (This process was repeated for 4-times ) [17].The resulting residue is washed with ethanol ,chloroform and then with diethyl ether to get off white a semi solid product (compound II). (Yield 79.5%,) ( $R_f=0.36$  benzene :methanol; 8:2 ).

### "Synthesis of 1,2-O-Isopropylidene- $\alpha$ -D-Xylo-Pentodialdo-1,4-Furanose" (III):-



Compound (II) (5g ,22.7mmol) dissolved in ethanol absolute (60mL) is added to a stirred aqueous solution of sodium period ate (4.9g ,22.7mmol, in distilled water 60mL) at 0<sup>0</sup> C,for 15min. Then ethylene glycol (0.5mL) is added, the mixture kept stirring at R.T. for 60min. then filtered and (40mL; H<sub>2</sub>O) is added. The product is extracted with (3x50 mL; ethyl acetate), the extracts dried over (Mg SO<sub>4</sub>), filtered off, to get a brown gummy product (compound III) after evaporating the solvent [ $R_f = 0.78$ ; yield = 60%].

### "Synthesis of Schiff's Bases Derivatives" (IV a – e) .

A mixture of compound (III) (1.0g ; 5.3 mmole) with appropriate amino compounds (5.3 mmole)in absolute ethanol (20ml) and 3drops of glacial acetic acid is refluxed for 24hrs . The completion of the reaction is followed by T.L.C. (benzene: methanol ; 8:2) and by evaporating the solvent, the residue should be recrystallized with absolute ethanol, and the characteristic physical properties of compounds (IV a – e) are shown in Table 1 .

**Table (1) Physical Properties of Compounds (Iva – e) :-**

Compound No.	Amino group	Molecular formula	Molecular weight	M. p C <sup>0</sup>	R <sub>f</sub>	Yield %	color
IV <sub>a</sub>	4-amino antipyrine	C <sub>19</sub> H <sub>23</sub> N <sub>3</sub> O <sub>5</sub>	373	188 – 190	0.76	85	Deep brown
IV <sub>b</sub>	4-Toulidine	C <sub>15</sub> H <sub>19</sub> NO <sub>4</sub>	277	118 – 120	0.70	78	Deep brown
IV <sub>c</sub>	4-amino benzoic acid	C <sub>15</sub> H <sub>17</sub> NO <sub>6</sub>	307	128 – 130	0.58	74	Brown
IV <sub>d</sub>	4-Nitro aniline	C <sub>14</sub> H <sub>16</sub> N <sub>2</sub> O <sub>6</sub>	308	124 – 126	0.72	69	Orange
IV <sub>e</sub>	3-amino phenol	C <sub>14</sub> H <sub>17</sub> NO <sub>5</sub>	279	225 – 227	0.66	79	Brown

### Results and Discussion:

D. Glucose in furanoid form (D. Glucofuranose) has five hydroxyl groups, and all these groups are active for classical reactions and modifications, D. Glucofuranose has been chosen as a starting material since it is readily available and comparatively in

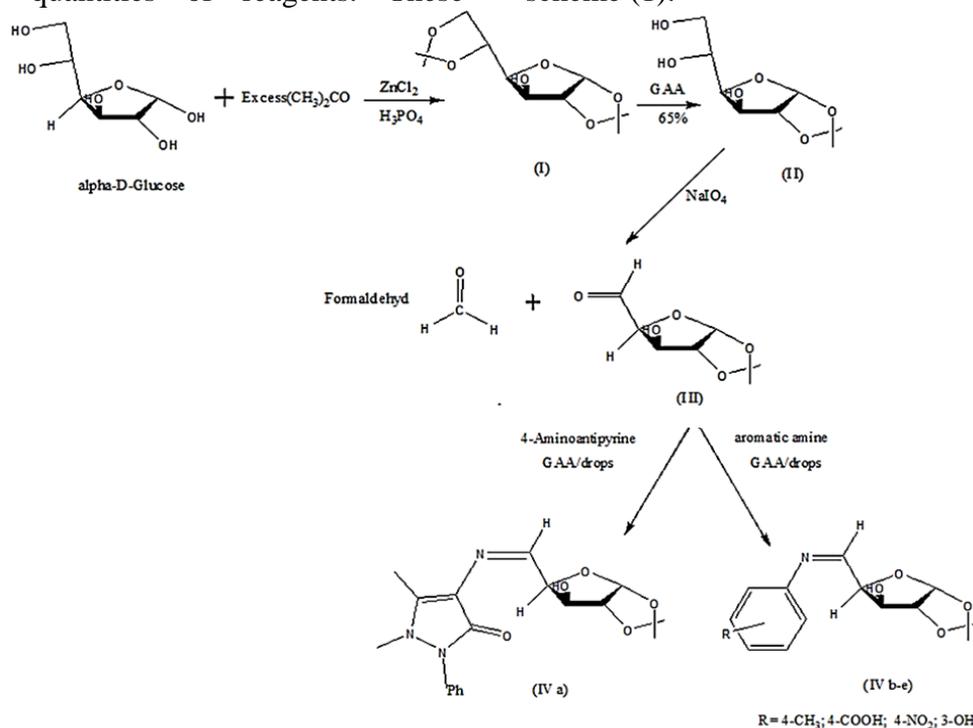
expensive compound. The synthesis of Schiff bases derivatives (IV<sub>a-e</sub>) needs first conversion of D – glucose into 1, 2: 5 , 6 – isopropylidene derivative (I). The acetal group is stable toward alkaline conditions but is readily hydrolyzed by dilute acids [18], thus it is very useful as a blocking agent , and

as used in this work for protecting of hydroxyl groups at C – 1, C – 2 , C – 5 and C – 6.

A number of procedures using different acid catalysts are available for the acetonation of D- glucose but, generally, they are time-consuming and require large quantities of reagents. These

methods include the use of mineral acids such as sulphuric acid [19] and Lewis acids such as ferric chloride [20] and Zinc chloride as catalyst .

The 1,2 : 5,6 – di – O – isopropylidene –  $\alpha$  – D – glucofuranose (I) is synthesized by using  $ZnCl_2$  catalyst [15], as shown in scheme (1):



Scheme (1)

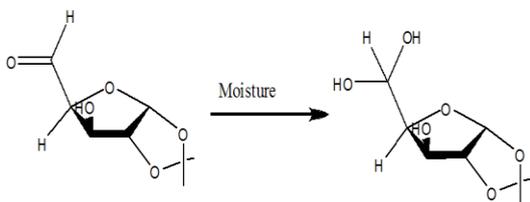
The Compound (I) is characterized by FT – IR spectrum which shows a strong band at  $(3431) \text{ cm}^{-1}$  for (OH) group in C – 3; a strong band at  $(2985) \text{ cm}^{-1}$  to 4(CH<sub>3</sub>) di-isopropylidene methyl groups; (C–H) bending for CH<sub>2</sub> and CH<sub>3</sub> groups appearance at  $(1458$  and  $1375) \text{ cm}^{-1}$  and the acetal (C – O – C) shows the bands  $(1247-1070) \text{ cm}^{-1}$ . The  $H^1$  – NMR spectrum exhibits the signals in  $\delta$  ppm. at:  $(1.229-1.374)$  (4S., 12H, isopropylidene protons);  $2.10$  (S., 1H, OH group);  $2.5$  (S., 6H, protons of solvent DMSO);  $3.382$  (d., 2H, H–6);  $3.856$  (t., 1H, H–3);  $4.086$  (t., 1H, H–4);  $4.220$  (t., 1H, H– 5);  $4.288$  (t., 1H, H – 2); and  $5.103$  (d., 1H, H – 1). The 1,2 – O – isopropylidene –  $\alpha$  – D – glucofuranose (II) is synthesized by using 65% GAA; an isopropylidene ring

may be hydrolyzed in acidic media easily as mentioned in the introduction , as shown in scheme (1).

The FT – IR spectrum of compound (II) shows a broad band at  $(3487-3228) \text{ cm}^{-1}$  for 3(OH) groups; and  $(2937) \text{ cm}^{-1}$  for 2(CH<sub>3</sub>) isopropylidene methyl group and  $H^1$  – NMR spectrum appearance the signals at  $(1.229-1.374)$  (2S., 6H, isopropylidene protons); and  $2.004$  (S., 3H, 3OH group).

1,2 – O – isopropylidene –  $\alpha$  – D – Xylo – pentodialdo – 1,4 – furanose (III), is prepared by using  $NaIO_4$ . Glycols (compounds contain two vicinal (OH) groups oxidized by periodate, which cleaves the carbon – carbon (bearing OH groups) bond and formation of two compounds containing carbonyl groups [21], show that in scheme(1).

The FT-IR spectrum of (III) give (3442–3388) $\text{cm}^{-1}$  a broad band for 3(OH) groups shows that some of the prepared aldehyde may exist in the hydrate form [22], as shown below:



The band at (2794) $\text{cm}^{-1}$  is due to (C–H) aldehydic, and the band at (1716)  $\text{cm}^{-1}$  is due to aldehydic carbonyl group. The  $\text{H}^1$ -NMR spectrum (III), shows the signals at: 2.008 (S., 3H, 3OH group). The data from the FT – IR and  $\text{H}^1$  – NMR spectra agree with the investigation about hydrate form of compound (III). The electronic (UV–Vis) spectrum displays an intense peak at  $\lambda$  max (DMSO) =302.0 nm; Abs.=2.386(DMSO) related to the ( $n \rightarrow \pi^*$ ) and ( $\pi \rightarrow \pi^*$ ) transitions of carbonyl group.

The final step in this work attempts to prepare the goal Schiff's bases derivatives ( $\text{IV}_{a-e}$ ) derived from the prepared aldehydic derivative (III) with appropriate amino compounds in equimolar dissolved in absolute alcohol in the presence of drops of glacial acetic acid [23].

Schiff base ( $\text{IV}_a$ ) can be prepared from the reaction between compound (III) and 4 – amino antipyrine as shown in scheme(1).

The FT – IR spectrum of ( $\text{IV}_a$ ) display the bands at (3398) $\text{cm}^{-1}$  for (OH)sugar group; (3064) $\text{cm}^{-1}$  for (C – H) aromatic ring; (2929) $\text{cm}^{-1}$  to aliphatic 4( $\text{CH}_3$ ) groups (2 for sugar moiety and 2 for antipyrine compound); 1737  $\text{cm}^{-1}$  to (C=O) carbonyl group for cyclic acetamide; (1651) $\text{cm}^{-1}$  for (C=N) (azomethane group); (1633) $\text{cm}^{-1}$ for (C=C) vinylic of antipyrine, (1593) $\text{cm}^{-1}$  for the (C=C) aromatic ring; and (758 +

698) $\text{cm}^{-1}$  to (C–H) bending vibration for mono substituted aromatic ring.

The  $\text{H}^1$ -NMR spectrum of ( $\text{IV}_a$ ) displays the signals at: 1.759 (S., 3H, =C–  $\text{CH}_3$ , methyl in antipyrine moiety); 2.377 (S., 3H, N –  $\text{CH}_3$ , methyl to anti pyrine moiety); [6.691–7.367] (multiplet, 5H, protons for benzene ring); and 7.478 (d., 1H, imine or azomethane group).

The (UV– Vis) spectrum of ( $\text{IV}_a$ ), shows an intense peak at  $\lambda$  max (DMSO) = 278.0 nm; Abs. = 2.023 (DMSO) due to the electronic transition ( $n \rightarrow \pi^*$ ) and ( $\pi - \pi^*$ ) for azomethane group.

Another series of Schiff's Bases ( $\text{IV}_{b-e}$ ) can be prepared from the reaction between the synthesis of aldehydic compound (III) with aromatic amines as follows in scheme (1). The goal of the new series of Schiff's bases ( $\text{IV}_{a-e}$ ) synthesized are listed in Table 2.

**Table (2): The Molecular and Structural Formula of Schiff's Bases ( $\text{IV}_{a-e}$ )**

Compound No.	Molecular formula	Structural formula
$\text{IV}_a$	$\text{C}_{19}\text{H}_{23}\text{N}_3\text{O}_5$	
$\text{IV}_b$	$\text{C}_{15}\text{H}_{19}\text{NO}_4$	
$\text{IV}_c$	$\text{C}_{15}\text{H}_{17}\text{NO}_6$	
$\text{IV}_d$	$\text{C}_{14}\text{H}_{16}\text{N}_2\text{O}_6$	
$\text{IV}_e$	$\text{C}_{14}\text{H}_{17}\text{NO}_5$	

The FT – IR of these derivatives ( $\text{IV}_{a-e}$ ) show the disappearance of (C=O) (aldehydic in 1716 $\text{cm}^{-1}$ ) for compound (III) and formation the (C=N) at frequencies range between (1683 –

1600) $\text{cm}^{-1}$ . These data are shown in Table 3 which display the ( $n \rightarrow \pi^*$ ) and ( $\pi \rightarrow \pi^*$ ) electronic transitions of the

formed (C=N-) group in azomethane and imine moiety of the formed derivatives.

**Table (3) The UV – Vis , and FT – IR Data for Compounds (IV<sub>b-e</sub>)**

Comp.no,	R	$\lambda_{\text{max}}$ nm.	Abs.	$\nu(\text{C}=\text{N})$ $\text{cm}^{-1}$	$\nu(\text{C}=\text{C})\text{cm}^{-1}$ Aromatic	$\nu(\text{C}-\text{H})\text{cm}^{-1}$ Aromatic	Other ( $\nu$ ) $\text{cm}^{-1}$
IV <sub>b</sub>	4-CH <sub>3</sub>	266.0	3.9	1683	1616;1516	3030	—
IV <sub>c</sub>	4-COOH	296.0	0.807	1604	1523;1490	3059	(OH); (C = O)Acid 3377; 1685
IV <sub>d</sub>	4-NO <sub>2</sub>	388.0	1.580	1600	1529;1481	3089	N – O 1506,1309
IV <sub>e</sub>	3-OH	212.0	2.18	1624	1614;1508	3039	OH 3375

The H<sup>1</sup>.NMR spectrum for (IV<sub>b</sub>); R= 4-CH<sub>3</sub>; exhibits the new signals in  $\delta$ ppm. at: (7.155–6.953) (multiplete, 4H, benzene ring protons); 7.500 (d., 1H, to imine proton or azomethane group) and to (IV<sub>d</sub>); R=4-NO<sub>2</sub>; show the new signals at:7.602 (multiplet., 4H, aromatic protons); 8.195 (d.,1H, imine proton).

All of the prepared derivatives (Schiff's bases) are characterized by (C.H.N) micro elemental analysis and the obtained results show a very good agreement between the found and the calculated percentages. The elemental analysis of the obtained results are shown in Table 4.

**Table (4) The C.H.N Data of the Prepared Derivative (IV<sub>a-e</sub>), Schiff 's Bases.**

Comp. No	Molecular Formula.	R	calculated			Found.		
			C%	H%	N%	C%	H%	N%
IV <sub>a</sub>	C <sub>19</sub> H <sub>23</sub> N <sub>3</sub> O <sub>5</sub>	—	61.12	6.16	11.26	60.54	6.56	12.00
IV <sub>b</sub>	C <sub>15</sub> H <sub>19</sub> NO <sub>4</sub>	-CH <sub>3</sub>	64.981	6.859	5.054	63.985	6.597	5.054
IV <sub>c</sub>	C <sub>15</sub> H <sub>17</sub> NO <sub>6</sub>	-COOH	58.631	5.537	4.560	57.934	5.506	4.231
IV <sub>d</sub>	C <sub>14</sub> H <sub>16</sub> N <sub>2</sub> O <sub>6</sub>	-NO <sub>2</sub>	54.545	5.194	9.0909	53.693	5.141	9.978
IV <sub>e</sub>	C <sub>14</sub> H <sub>17</sub> NO <sub>5</sub>	-OH	60.21	6.09	5.01	59.26	6.20	5.66

#### Biological Activity:-

Organic compounds that contain heterocyclic moiety can be considered as important classes of compound having a wide range of biological activity. The synthesized derivatives such as (III and IV<sub>a-e</sub>) in this work are expected to possess a biological activity since they have an active moiety like Schiff 's base. When we use the antibiotics tested with concentration equal to that of the chemical tested, no inhibition of growth for any microorganism is noted so we have to use a concentration fraction of antibiotics (standard) higher than that of the tested chemicals to ensure inhibition.

In this work, the performance of antibacterial test has been achieved as in the disc diffusion method [24]. (III and IV<sub>a-e</sub>) derivatives are assayed for their antimicrobial activity *in vitro* against two of each strain of negative and positive grams bacteria like (*Escherichia coli*, *pseudomonas aeruginosa*, *staphylococcus aureus*, *Bacillus subtilis*) and fungi like *candida*. The previous bacteria are activated in nutrient growth medium at 37°C for 24 hour.

All the synthesized compounds (III and IV<sub>a-e</sub>) are evaluated for their antimicrobial and anti fungi activities. The activity of compounds is determined by measuring the diameter

of the empty region around the well (Inhibition zone). The results show that most of the tested compounds possess a

good antibacterial activity as listed in Table 5:

**Table (5) Anti – Bacterial and Fungi Activity for some of the Synthesized Compounds.**

Comp. No.	<i>E coli</i>	<i>pseudomonas</i>	<i>Staphylococcus aureus</i>	<i>Bacillus</i>	<i>candida</i>
Control.	—	—	—	—	—
III	10mm	12mm	10mm	16	—
IV <sub>a</sub>	11	11	11	9	—
IV <sub>b</sub>	—	14mm	16	—	10
IV <sub>c</sub>	10	12	13	12	—
IV <sub>d</sub>	11	9	11	10	—
IV <sub>e</sub>	—	12	—	—	—

**Key of symbols :-**

Inactive = inhibition zone = 5mm

Moderately active = (9 – 12 mm)

Slightly active = (6 – 9 mm)

Highly active = (13 – 17 mm)

The results of bioactivity, obtained from the general lab. Biology Department, College of Science- University of Baghdad, show the following data :

1. Compound III shows the highest antimicrobial against *Bacillus* and moderately activity towards (*E.coli*, *pseudomonas*, *staphylococcus aureus*).
2. Compounds (IV<sub>a-d</sub>) display the appearances of the moderately against *E. coli* bacteria, but the IV<sub>e</sub> do not show any biological activity towards the same bacteria.
3. compound IV<sub>b</sub> show a high activity toward the *Pseudomonas* and the others (IV<sub>a,c,d,e</sub>) show moderately activity toward the same bacteria.
4. Compounds (IV<sub>b,c</sub>) show the highest antimicrobial activity toward *staphylococcus* equal to (16+13) respectively, while the others (IV<sub>a,d</sub>) show moderately activate, but the IV<sub>e</sub> does not show any bioactive result to the same.
5. Compounds (IV<sub>a, c, d</sub>) shows the moderately bioactivity against *Bacillus*, but (IV<sub>b,e</sub>) do not show any activity toward the same bacteria.
6. Finally only compound (IV<sub>b</sub>) shows moderate bioactive action to the fungi (*candida*), but the others prepared in this work, do not show any bioactivity toward *candida*.

**Conclusions:**

1. In a previous study, a new series of Schiff bases containing sugar moiety is synthesized by the reaction of aldehyde prepared (III) and amino compound like 4-amino antipyrine and some of primary aromatic amino compounds in ethanol absolute.
2. The physical properties, spectral data, and C.H.N analysis give a good information of the suggested structures for the new goal compounds.
3. The antimicrobial activity of the prepared compounds (III and IV<sub>a-e</sub>) is tested against four bacterial and one fungi strains.
4. The bioactivity shows differs inhibition zone depending upon the amino compounds that joined with the monosaccharide moiety.

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## تحضير وتشخيص ودراسة الفعالية البايولوجية لبعض المشتقات الجديدة من قواعد شف المحتوية على مكونة السكريات الاحادية

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### الخلاصة:

يتضمن البحث تحضير سلسلة جديدة من مشتقات قواعد شف لسكر (  $\alpha$ -D-glucose ) ولقد تم تشخيصها مع دراسة فعاليتها البايولوجية. مجاميع الهيدروكسيل على ذرات الكربون (1,2&5,6) الموجودة على مكونة السكر تم تحويلها الى شكل الاسيتال من خلال التفاعل مع الاسيتون الجاف وحامض الفوسفوريك وكلوريد الخارصين اللامائي كعوامل مساعدة لإنتاج المشتق رقم (I). حلقة الاسيتال الخماسية للكربون(5,6) تم تحليلها باستخدام حامض الخليك الثلجي(65%) والتفاعل للناتج الجديد مع بيرأيودات الصوديوم تم إنجازه ليعطي مكونة الالديهيد والتي تم استخدامها لإنتاج سلسلة جديدة من قواعد شف من خلال التفاعل مع مركبات أمينية مختلفة ومنها 4-أمينو أنتي بايرين.

التراكيب الكيميائية المقترحة للمركبات المحضرة تم التثبت منها باستخدام أطيف الأشعة فوق البنفسجية، الأشعة تحت الحمراء والرنين النووي المغناطيسي البروتوني (H-NMR) وتحليل العناصر (C.H.N). وأن معظم المركبات المحضرة أظهرت فعالية كمضادات للبكتريا.

الكلمات المفتاحية: تحضير، الفعالية البايولوجية، قواعد شف.