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Synthesis of Some New Nucleoside Analogues from Theobromine via Schiff Base

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Abstract: ***Of the approximately 40 antiviral drugs formally approved for use, half of them are nucleoside or nucleotide analogues. So new derivatives of nucleoside analogues were obtained from the work that was conducted in this research. Firstly; the reaction of theobromine was reacted with sodium hydride in DMF at (0 °C) to give it's salt, then the produced salt was reacted with chloroacetyl chloride to produce 1-(chloro acetyl) theobromine [3]. Thereafter, compound [3] was reacted with hydrazine hydrate to give 1-(1-acetohydrazide) theobromine [4] then reacted with a different substitution of an aromatic aldehyde in absolute ethanol to give Schiff's bases derivatives [5-12]. The reaction of compounds [5-12] with 1,2:3,4-Di-O-isopropylidene- α- D- galactopyranosyl bromide [2] forming blocked nucleoside analogues [13-20]. Deblocking of these nucleoside using in 50% aqueous acetic acid to give the free nucleoside [21-28]. The new prepared compounds were identified by [FTIR, ^{1}H-NMR and ^{13}C-NMR] and their physical properties were measured. Furthermore, we have evaluated the effect of some prepared compounds on some bacterial and fungal strains.***

Keywords: Theobromine, Schiff base, anti-microbial

1. Introduction

Theobromine is also known as xanthine is a bitter alkaloid of the cacao plant, with the chemical formula $C_7H_8N_4O_2$ [1,2].Despite its name, the compound contains no bromine and word theobromine is derived from theobromine (from Theobroma cacoa; theo = god, and broma = food ; therefore food of the gods) and caffeine are the two most abundant methylxanthines in chocolate [3,4], both of which have received considerable attention in the food and nutrition fields, in part because of the physiological effects which they elicit [5].Theobromine is identified with pharmacological and biological activities of living organisms.[6,7-10]Theobromine has been used as a drug for its anticarcinogenic effect [11] , as a skin moisturant,[12] vasodilator,[13] treatment of Asthma,[14] antitussive,[15] cardiovascular,[16,17] anti-inflammatory[18] and as antimicrobial[19]. And also has been used to treat high blood pressure[20]. Theobromine is characterized as an antioxidant, prooxidant[21], and bronchodilator [22,23]. The original nucleoside analogues were synthesized in 1914 by Fischer[24] through condensation of tetra –*O*- acetyl – *α*-D-gluco pyronosyl bromide with silver salts of purines. Johnson and Hilbert[25], as well condensed the similar sugar with 2,4 dialkoxy pyrimidines in 1930. At this time, repeated and modified for these methods are uses[26].Three general Methods in Nucleoside Synthesis: Metal Salt (Koenigs-Knörr) Method[27], The fusion method[27] and Hilbert−Johnson and Silyl-Hilbert−Johnson[28].Schiff bases, they are prepared by the condensation between primary amine and active carbonyl compounds such reactions could be accelerated by base or acid catalysis or by heating.[29] As far as the use of acid catalyst is required[30,31], mineral acids, like H_2SO_4 or HCl, organic acids such as *p*-toluene sulphonic acids or pyridinium *p*-toluene sulphonate, acid resin, montmorillonite or even Lewis acids like $ZnCl_2$, $TiCl_4$, $SnCl_4$, BF_3Et_2O, $MgSO_4$, $Mg(ClO_4)_2$, etc., have been reported[32,33].Aslihan Yilmaz Obali and Halil Ismet Ucan[34] were reported novel dipodal Schiff base compound.There are numerous publications covering the use of Schiff bases in therapeutic or biological applications either as potential drug candidates or diagnostic probes and analytical tools[35,36].

2. Experimental

Materials and Methods

All chemicals used were supplied by: Merck, BDH, Fluka and sigma Aldrich chemicals companies. The melting point was recorded using Gallenkamp, electro-thermal melting point apparatus. Infrared spectra were recorded using (FTIR) 8400s Fourier transitions infrared spectrometer shimadzu, Japan, (KBr) disc in (4000-600) cm^{-1} spectral range, in the Department of Chemistry, College of Science, University of Baghdad and Research Laboratory. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on near magnetic resonance Bruker, Ulter-shield (400) MHz in(Isfahan University of Technology (IUT), Iran), DMSO-*d6*was used as a solvent. The biological activity was screened in the central environmental laboratory in college of science in university of Baghdad.

Preparation of 1,2:3,4-Di-*O*-isopropylidene- α- D-galactopyranose [1] [37a]

Freshly fused, and powdered anhydrous zinc chloride (9.5 g, 70 mmol) was rapidly weighed into a dry (250 ml) Erlenmeyer flask, and (100 ml) of dry acetone was added with stirring at room temperature until the zinc chloride was dissolved. Concentrated sulfuric acid (0.32 ml) was rapidly added drop-wise. To the resulting colorless solution finely powdered anhydrous D- galactose (9 g, 50 mmole) was added. The mixture was stirred magnetically for 4 hours. After that a suspension of (16 g) of anhydrous sodium carbonate in (28 ml) of water was added in portionsand the mixture was stirred for about one hour. The suspension was filtered, and theprecipitate was washed several times with acetone. Thefiltrate and washings are combined, then the solution was evaporatedunder reduced pressure. The mixture was extracted 3 times with ether (3×10ml), dried withanhydrous sodium sulfate, filtered, and evaporated to dryness underreduced pressureto give product [1] as pale yellow syrup, (9.54 g, 73.37%). Compound [1] showed physical properties according reference above.

Preparation of 1,2:3,4-Di-*O*-isopropylidene- α- D-galactopyranosyl bromide [2] [37b]

Hydrogen bromide in glacial acetic acid (5 ml) of (45%) was added to 1,2:3,4-*di*-*O*-isopropylidene- α- *D*-galactopyranose [1] (4 g) then (5 ml) of glacial acetic acid was added. The mixture was stirred for 30 min. at room temperature and then left for 6 hours at room temperature. The mixture left over night at (5˚C) then the mixture was dissolved in chloroform and neutralized with saturated aqueous sodium bicarbonate solution and extracted with chloroform (4× 8ml), dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to give a brown syrup (3.54 g, 71.23%).

Synthesis 1-(chloro acetyl) theobromine[3][38]

The title new compound synthesized according to literature[250] with modification. Chloroacetyl chloride (0.124 g, 0.001mol) was added dropwise with stirring to a mixture of theobromine (0.2 g, 0.001 mol) and NaH (0.026 g, 0.001 mol) in DMF (15 ml) at 0-5 °C with stirring for 1 h. in ice cool and continue stirring for 5 h. at room temperature. The solvent was evaporated under reduced pressure to give [3]. (0.1 g, 71 %)

Synthesis of 1-(1-acetohydrazide) theobromine.[4][39]

To the compound [3] (0.5 g, 0.0019 mol), hydrazine hydrate (0.0038 mol) was added and kept for reflux around 6 hours in DMF as a solvent. The solvent was evaporated; the product obtained was filtered, washed with water and dried. (0.4 g, 81.6%)

Preparation of Schiff Base Derivatives [5-12][40]

To a hot stirred solution of the hydrazide [4] (1 gm, 0.004 mol.) in DMF (10 ml.) appropriate aromatic aldehyde (0.004 mol.) was dissolved in ethanol containing 3-4 drops of glacial acetic acid. The reaction mixture was heated to (90-110)^{0}C for (4-6) hrs. The separated solid was put in ice water then filtered, recrystallized from ethanol. Physical properties of the products are listed in Table (2)

General procedure for Synthesis of Protected Nucleoside Analogues.[41] [13-20]

Schiff base derivatives (0.0012 mol) were finely powdered and suspended in (20 ml) sodium-dried m-xylene in the presence of celite (1 g) and to remove traces of water isotrop the solvent was partially distilled off. When telling the temperature raised to 137 ^{0}C, then cool (below 50^{0}C). The protected sugar [2] (0.5 g, 0.0012mol) in dried m- xylene (30ml) was added and refluxed with vigorous stirring for 1 h. TLC (chloroform-ether 9:1). Indicated the completion of the reaction then filtered from the hot xylene, suspension washed with dichloro methane (6 ml). The organic layer was washed with (3x5 ml) of 20% aqueous potassium iodide to remove traces of the mercuric salt, washed with water (3x5 ml) dried over anhydrous sodium sulphate and the solvent was removed to giveprotected nucleoside analogues [13-20]. Physical properties of these compounds were listed in the table (3)

Hydrolysis of protected Nucleoside Analogues.[42][21-28]

For galacto nucleoside [13-20] a solution of blocked nucleoside (0.0003mol.) in (21 ml) of 50% aqueous acetic acid was refluxed for 15 min. (10 ml) of water was added and extracted with chloroform (3 x 5 ml), then dried over anhydrous Na_2SO_4, the solvent was removed to give the free nucleoside [21-28]. Physical properties of these compounds were listed in the table (4).

Anti-microbial activity test

The inhibition zone of growth of microorganisms was measured against *Staphylococcus aureus* and *Streptococcus* (Gram +ve) and *Escherichia coli* and *Pseudomonas aeruginosa* (Gram –ve) and *Aspergillus Flavus* (fungal) using Cup-plate methods. The petri dishes were placed on a flat surface to ensure that the layers of the medium were of uniform thickness. Cylindrical cavities of 6 mm dimeter were made on the medium. 50 µl of the test and standard solutions were transferred into cylindrical cavities, the plates were incubated for 24 h. for bacteria and 72 h. for fungal, at 37^{0}C and the circular inhibition zone was measured.

Table 1: The physical properties and the Fourier infrared values

Comp. NO.	Physical properties				FTIR absorption cm^{-1}			
	Comp. structure	Melting point °C	Yield %	Color	ν(N-H)	ν(C=N)	ν(C=O)	Others
2		syrup	71.23	brown	-	-	-	601 for ν(C-Br)
Theo.		356	-	White	3448	1695 1670	1595	ν (C-H aliph.) 2952 2885

3		220-222	71	light brown	-	1743, 1691, 1631	1554	ν (C-H aliph) 2964 2842 ν(C-Cl) 775.3
4		110-112	81.6	Brown	3247	1693 1631	1525	$\nu(NH_2)$ (asy.3457) (sy.3396)

Table 2: The physical properties and the Fourier infrared values

Comp. NO.	Physical properties				FTIR absorption cm^{-1}			
	Comp. structure	Melting point °C	Yield %	Color	ν(N-H)	ν(C=O)	ν(C=N)	Others
5		202-206	91	Off white	3425	1776 1710,1625	1600 1585,1548	1099 for ν(C-Cl)
6		Oily	75	Yellowish-brown	3326	1699 1677	1618 1573	ν(O-H) 3417
7		Oily	78	Yellow	3367	1689 1620	1579 1550	ν(O-H) 3455
8		173-176	89	Pale brown	3438	1703 1635	1608 1587	ν(C-Br) 1068
9		210	90	Reddish-orange	3492	1687	1602,1548	-
10		124-118	81	Off white	3433	1687,1622	1602,1575	ν(C-O-C) 1026,1217
11		118-125	86	Off-white	3356	1687 1623	1598 1579	ν(C-O-C) 1261,1018
12		182-187	89	Pale yellow	3361	1772,1726 1698	1623 1577	ν (NO2) 1353,1525

Table 3: The physical properties and the Fourier infrared values

Comp. NO.	Physical properties				FTIR absorption cm^{-1}			
	Comp. structure	Melting point °C	Yield %	Color	ν (C-H) aromatic	ν(C=O)	ν(C=N)	Others
13		303- 301	66	Pale brown	3091	1683, 1616	1583, 1546	ν (C-Cl) 1139
14		Syrup	53	Yellow	3045	1686	1574	(O-H) 3332-3450
15		Syrup	58	Pale brown	3032	1678, 1614	1562	(O-H) 3240-3482
16		267- 265	52	Brown	3083	1668, 1610	1583	ν (C-Br) 1140
17		227- 229	74	Yellowish-orange	3009	1681, 1650	1602, 1523	-
18		Syrup	63	Yellow	3002	1691-1602	1569, 1512	ν (C-O-C) 1027,1251
19		Syrup	60	Off white	3062	1681, 1622	1598, 1581	ν (C-O-C) 1018,1238
20		196- 193	51	Yellow	3074	1683,1625	1558	ν (NO_2) sy. 1335, asy.1530

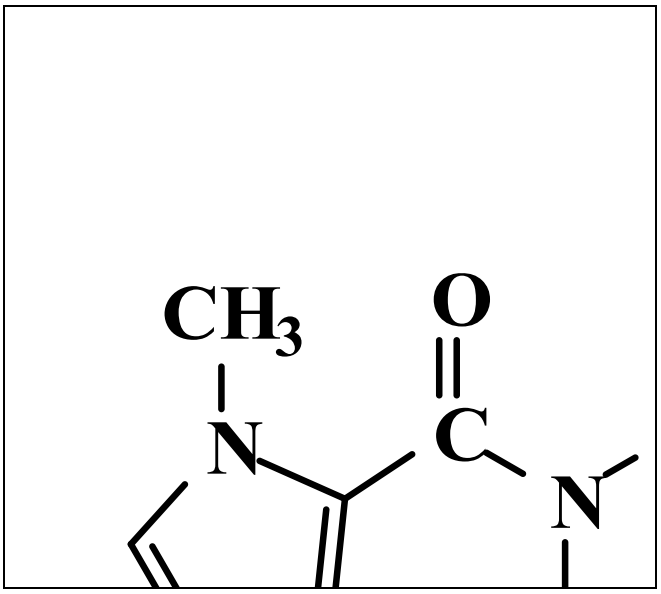
Table 4: The physical properties and the Fourier infrared values

Comp. NO.	Physical properties				FTIR absorption cm^{-1}			
	Comp. structure	Melting point °C	Yield %	Color	ν (O-H)	ν (C-H) aromatic	ν(C=O)	Others
21		Dec. 283	89	Off white	3442,3223	3018	1645,1616	ν (C-Cl) 1135
22		Syrup	92	Pale Yellow	3465,3258	3037	1665	-

23		Syrup	89	Pale yellow	3412-3387	3033	1674,1622	-
24		230- 234	82	Off white	3438,3296	3028	1674,1623	ν (C-Br) 1131
25		198- 201	88	Pale orange	3465	3020	1677,1604	-
26		Syrup	78	Off white	3429-3279	3053	1686-1612	ν (C-O-C) 1243,1015
27		Syrup	85	Brown	3485,3431	3004	1679,1623	ν (C-O-C) 1265,1018
28		Dec. 173	88	Yellow	3326-3215	3051	1683,1625	ν (NO_2) sy. 1532,asy.1331

3. Result and Discussion

Current work has been going to the building of new nucleoside analogueswith modified nucleobase and sugar moiety which synthesized for the first time, by pairing between theobromine as a nucleobase and a sugar moiety such as (galacto bromo protected sugar), which gives after hydrolysis in acidic medium, our main goal the free nucleoside analogues. As shown in the scheme (1)

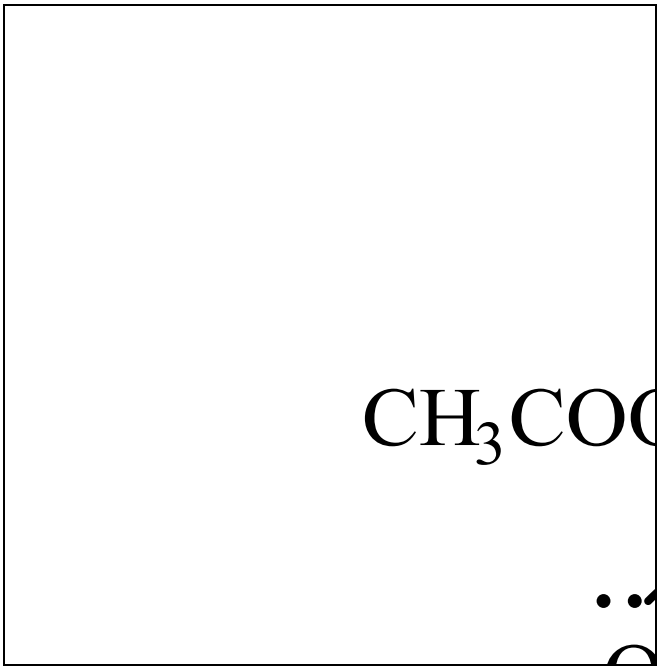


In order to modify theobromine it was converted to 1-(chloro acetyl) theobromine [3] via reaction theobromine with chloro acetyl chloride. This reaction carried out with sodium hydride in dry dimethylformamide (DMF) at (0 °C) to give theobromine salt in situ which after that forming the 1-(chloro acetyl)theobromine [3]. FTIR spectrum of compound [3] showed the appearance of absorption band at (1743) cm^{-1} due to ν (C=O) of acetyl group in addition of theobromine carbonyl [43] and (3029) cm^{-1} for ν (C-H aromatic), (2925 and 2827) cm^{-1} due to ν (CH_3). The disappeared band of (NH) band gives a good evidence for formation of compound [3]. Other data listed in Table (1).

Compound [3] was reacted with hydrazine hydrate (95%) in DMF to give hydrazide derivative [4] (scheme 1) FTIR spectrum of compound [4] showed the appearance of absorption band at (3457, 3396 cm^{-1}) due to asymmetric and symmetric ν (NH_2). While stretching bands at (3247 and 1693 cm^{-1}) for (NH and C=O amide) respectively. FTIR data of compounds [4] were listed in Table (1).

Compound [4] was treated with different substituted aromatic aldehydes to form the Schiff's bases [5-12] (scheme1). The FTIR spectra of compounds [5-12] showed absorption bands at (3492-3309) cm^{-1} due to ν (NH) and appearance band at (1629-1548) cm^{-1} due to ν (C=N) of Schiff's bases and (1776-1612) cm^{-1} due to ν (C=O amide). Also, absorption bands were shown at (3082-3002) cm^{-1} for ν (C-H aromatic) and at (2991-2815) cm^{-1} for aliphatic groups. All data of the FTIR spectra of compounds [5-12] were listed in Table (2). The ^{1}H-NMR spectrum of compound [8, 12] showed a singlet signal at δ= (8.67-8.9) ppm due to (N=CH) proton, a singlet signal at δ= (7.69-8.74) ppm due to (-NH) proton and disappearance of the signal of (NH_2) proton as shown in table (5).

The mechanism of Schiff's bases [44] was formed firstly by polarization of aldehyde carbonyl and secondly by nucleophilic attack of the NH_2 lone pair, in theobromine hydrazide, on polarized carbonyl group forming an ammonium intermediate which after proton transfer and dehydration gives an iminum intermediate in third and fourth steps while in five steps give Schiff bases after deprotonation through abstraction the proton by acetate anion. The all steps of mechanism are shown in Scheme (2).



We synthesized for the first time new nucleoside analogues containing galactopyranoside. To obtain synthetic target the new protected nucleoside analogues following the Koenigs-Knörr condensation method by coupling of bromo isopropylidine galactose [2] with theobromine Schiff bases [5-12], through nucleophilic substitution afforded new blocked nucleoside analogues [13-20]. The FT-IR spectrum for compounds [13-20], showed disappearance of stretching (N-H) for hydrazone derivatives, also showed stretching bands between (3091- 3002) cm^{-1} for stretching (C-H) aromatic and at (2974-2825) cm^{-1} due to stretching (C-H) aliphatic. Stretching band between (1602-1550) cm^{-1} refer to stretching (C=N), while stretching bands between (1484-1421) cm^{-1} were attributed to stretching (C=C).The ^{1}H-NMR spectrum of compound [18] in δ ppm, table (7), showed a singlet signal at (2.03) ppm for ($4CH_3$) of isopropylidine, while at (3.785) due to (OCH_3,$2NCH_3$,CH_2,H_6',H_6''). While multiplet signals between (3.96-5.87) due to other sugar protons (5H). Multiplet signals between (6.98-8.1) were due to aromatic protons. Singlet signal at (8.63) referred to (CH=N benzylidine), while singlet signals at (11.36) referred to imidazole**.** The ^{13}C-NMR spectrum of compound [18] showed a signals at (22.15) for two methyl of theobromine (2CH3); also a signal between (55.22-55.63) ppm for ($4CH_3$) of isopropylidine and (OCH_3). A signal at (58.03) refers to (CH_2). A signal between (62.53-84.12) refers to sugar carbon. While the signals between (113.9-130.2) refers to aromatic carbons. The signal at (144.83) refer to benzylic, a signal between (147.43-160.55) due to three imidazole carbon. The signal at (161.68) belongs to C=O amide.The C=O of theobromine appeared at (164.6,169.9), spectral data are listed in table (8).

The isopropylidene protecting group in galactose moiety [13-20] was hydrolyzed using 50% aqueous acetic acid afforded our target the free nucleoside analogues [21-28]. The free nucleoside analogues [21-28] were identified by FT-IR. The FT-IR spectra of compounds [21-28] showed the appearance of broad bands between (3485-3215) cm^{-1} for hydroxyl group of a sugar moiety which give a good evidence for success and complete hydrolysis of nucleoside analogues. Stretching bands between (3087- 3004) cm^{-1} belong to υ (C-H) aromatic while stretching bands between (2997-2815) cm^{-1} belong to υ (C-H) aliphatic. Stretching bands at (1697-1604) cm^{-1} and at (1484-1419) cm^{-1} belong to υ (C=O) and υ (C=C) aromatic respectively. All FT-IR data were listed in table (4). The ^{13}C-NMR spectrum of compound [25] showed signals between (24.2-38.8) for two methyl of theobromine (2CH3); a signal at (60.55) refers to (CH_2). A signal between (67.1-85.66) refers to sugar carbon. While the signals between (111.65-129.46) refers to aromatic carbons. The signal at (145.5) refer to benzylic, a signal at (145.61) due to imidazole carbon. The signal at (151.93) belongs to C=O amide.The C=O of theobromine appeared at low field (171.3-179.6),spectral data are listed in table (9).

Table 5: ^{1}H–NMR – Spectral data (δ ppm) for compounds

No.	Compounds	δ ppm
8		3.39 (s,6H,$2CH_3$); 3.84 (s,2H,CH_2) ; 7.61-8.02(m,4H , aromatic) ; 8.11-8.19(s,1H,NH); 8.67(s,1H,CH=N benzylidine); 11.54(s,1H,CH imidazole).

12		3.32(s,6H ,2CH_3); 3.84(s,2H,CH_2); 7.69-8.74(m,5H , aromatic and NH) ; 8.9 (s,1H,CH=N benzylidine); 11.75(s,1H,CH imidazole).

Table 6: ^{13}C–NMR – Spectral data (δ ppm) for compounds

No.	Compounds	δ ppm
8		29.8,32.52(2C,2CH_3); 46.62 (1C,CH_2); 123.06-131.96(6C, aromatic); 133.15(1C, benzylidine);133.2-146.4(3C,imidazole);157(1C,C=O amide); 164.88,165.01(2C,C=O Theo)
12		29.1,33.03(2C,2CH_3); 49.9(1C,CH_2); 120.62-135.71(6C, aromatic); 142.69(1C,benzylic); 145.29-157.48(3C,imidazole); 160.48(1C,C=O amide); 165.145,165.279(2C,C=O Theo).

Table 7: ^{1}H–NMR – Spectral data for compound[18]

No.	Compounds	δ ppm
18		2.03(12H, 4 CH_3 isopropylidine); 3.785(s, 13H, OCH_3, 2 NCH_3, CH_2, H'_6 ,H''_6); 3.96-5.87(m,5H,sugar protons); 6.98-8.1(m,4H,aromatic); 8.63(s,1H, CH=N benzylidine); 11.36(s,1H,C-H imidazole).

Table 8: ^{13}C–NMR – Spectral data for compound[18]

No.	Compounds	δ ppm
18		22.15(2C,2CH_3 theo); 55.22-55.63(5C, 4 CH_3 isopropylidine &OCH_3); 58.03(1C, CH_2); 62.53-84.12(6C,sugar); 113.9-130.2(6C,aromatic); 144.83(1C,benzylic); 147.43-160.55(3C,imidazole); 161.68(1C,C=O amide) ;164.6,169.9(2C,C=O theo).

Table 9: ^{13}C–NMR – Spectral data for compound[25]

No.	Compounds	δ ppm
25		24.2-38.3 (6C,2CH_3); 60.55(1C, CH_2); 67.1-85.66(6C,sugar); 111.65-129.46 (6C,aromatic); 145.5(1C,benzylic); 145.61(3C,imidazole); 151.93(1C,C=O amide); 171.3-179.6(2C,C=O theo).

Biological Activity of Some of Prepared Compounds

Microbiological Test: Some of the synthesized compounds were screened in vitro against four types of bacteria including, *staphylococcus* aurus and *streptococcus* as gram positive and *E.coil* and *pseudomonasauroginosa* as gram negative, also screened antifungal activity against

Aspergillus Flavus. The obtained results are listed in table (10)

Table 10: Inhibition Zones of some newly synthesized compounds

Comp. No.	Gram negative bacteria		Gram positive bacteria		Fungal
	E-Coli	Pseudo.	Stap.	Strept.	Asper.
Control	-	-	-	-	-
13	-	4	8	7	-
14	7	-	5	6	5
23	12	12	11	3	12
26	-	11	12	5	11

Solvent: DMSO (used as control) ; [C]=0.004 mole
Zone of inhibition: (-) no inhibition zone; (3-6) weak; (7-10) moderate; (11-20) strong.

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