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SYNTHESIS AND BIOLOGICAL ACTIVITY EVALUATION OF NEW NUCLEOSIDE ANALOGUES FROM THEOBROMINE LINKED TO SCHIFF BASE

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ABSTRACT
Most of antiviral and antitumor drugs that have been world-wide used belong to the nucleoside analogues. For this reason, a series of new nucleoside analogues were synthesized, using theobromine as a nucleobase, for the first time, by conversion to it[s] chloro acetyl derivative[3], which was treated with hydrazine hydrate to give the key intermediate[4]. Condensation of compound[4] with different aromatic aldehyde gave Schiff base[5-10] then they were subjected to condens with 1-bromo tetraacetyl glucose [2] forming blocked nucleoside analogues[11-16]. Deblocking of these nucleoside using methanolic sodium methoxide afforded our target the free nucleoside [17-22]. The synthesized compounds were identified by FT-IR spectroscopy and some of them with ^{1}H-NMR and ^{13}C-NMR spectroscopy.

KEYWORDS: Theobromine, Schiff base, anti-microbial.

INTRODUCTION

Nucleoside analogues, together synthetic and of natural product source, have been broadly used in medicine [1], especially as antiviral, anticancer agents [2-4] with antimicrobial and cholinesterase inhibitory activities [5-8] hepatitis B virus [9-13], hepatitis C virus[14-17], herpes simplex [18,19], Human Immunodeficiency Virus (HIV) and neoplasms [20'21] some nucleoside analogues used to treat Acquired Immune Deficiency Syndrome (AIDS) infections[22]. Theobromine (TBR) is a common known alkaloid and it is found in structures of cacao, tea, coffee [23] and chocolate [24]. Synthesis of Theobromine Derivatives by Davir González-Calderón *et al*. [25] Synthesized caffeine from theobromine by modification of the Pavia's synthesis procedure, The N-alkylation of theobromine using methyl iodide in methanolic sodium methoxide solution allows an efficient synthesis of caffeine. Schiff bases contain the imine group (-C=N-), also known (azomethine or imine) are a nitrogen analogues the aldehyde or ketone in which the carbonyl group (C=O) is replaced by an azomethine or imine group [26]. Schiff bases, widely used in organic synthesis chemistry, had been known to chemists for a long time. Schiff bases are important intermediates for the synthesis of various bioactive products [27]. Schiff bases are present in various natural, semi-synthetic, and synthetic compounds and have been demonstrated to be essential for their biological activities[28,29].

MATERIALS & METHODS

All chemicals used were supplied by: Merck, BDH, Fluka and sigma Aldrich chemical 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 the college of science in university of Baghdad.

Preparation of Beta-glucose Penta acetate [1] [30]

A mixture of glucose (5 g, 27.75 mmol), sodium acetate anhydrous (4 g, 48.76 mmol) and acetic anhydride (25 ml) place in a 100 mile round bottomed flask fitted with a condenser. The mixture was heated on a water bath until a clear solution is obtained (approximately 30 minutes), the mixture was shaken from time to time. Then heating continues for 4 hrs. The reaction mixture is poured into (50 ml) of ice water in a beaker. The crystal was filtered at the pump and wash well with water. The product [1] is recrystallize from ethanol. (MP 131-132) (9.3 g, 85.92%).

Preparation of α-Bromo glucose tetra acetate [2] [31c]

The acetylated glucose [2] (0.5 g, 1.3mmole) was treated with 50% hydrogen bromide in glacial acetic acid (2 ml) (which was added drop wise at 0^0C. The solution was kept at 0^0C until TLC indicated reaction completion (generally within one hour) then poured into an ice-cold chloroform (17 ml), washed with iced water (3×15 ml) and then with a saturated aqueous solution of sodium bicarbonate to remove the remaining acid. After a final washed with iced water (20 ml) the organic layer was dried over anhydrous sodium sulfate and the solvent was evaporated to give colorless syrup (0.44 g, 84.62%). The product was used directly for the nucleoside synthesis.

Synthesis 1-(chloro acetyl) theobromine [3] [32]

The title new compound synthesized according to literature[32] with modification. Chloroacetyl chloride

(0.124 g, 0.001mol) was added drop wise 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 water, then continued stirring for 5 h. at room temperature. The solvent was evaporated under reduced pressure. The solid was filtered (0.1 g, 71%)

Synthesis of 1-(1-acetohydrazide) theobromine [4] [33]

To the compound [3] (0.5 g,0.0019mol), hydrazine hydrate 95% (0.0038 mol) was added and kept for reflux around 6 hours in (10 ml) DMF. The solvent was evaporated; the product obtained was filtered, washed with water and dried. (0.4 g, 81.6%)

Preparation of Schiff base derivatives [5-10] [34]

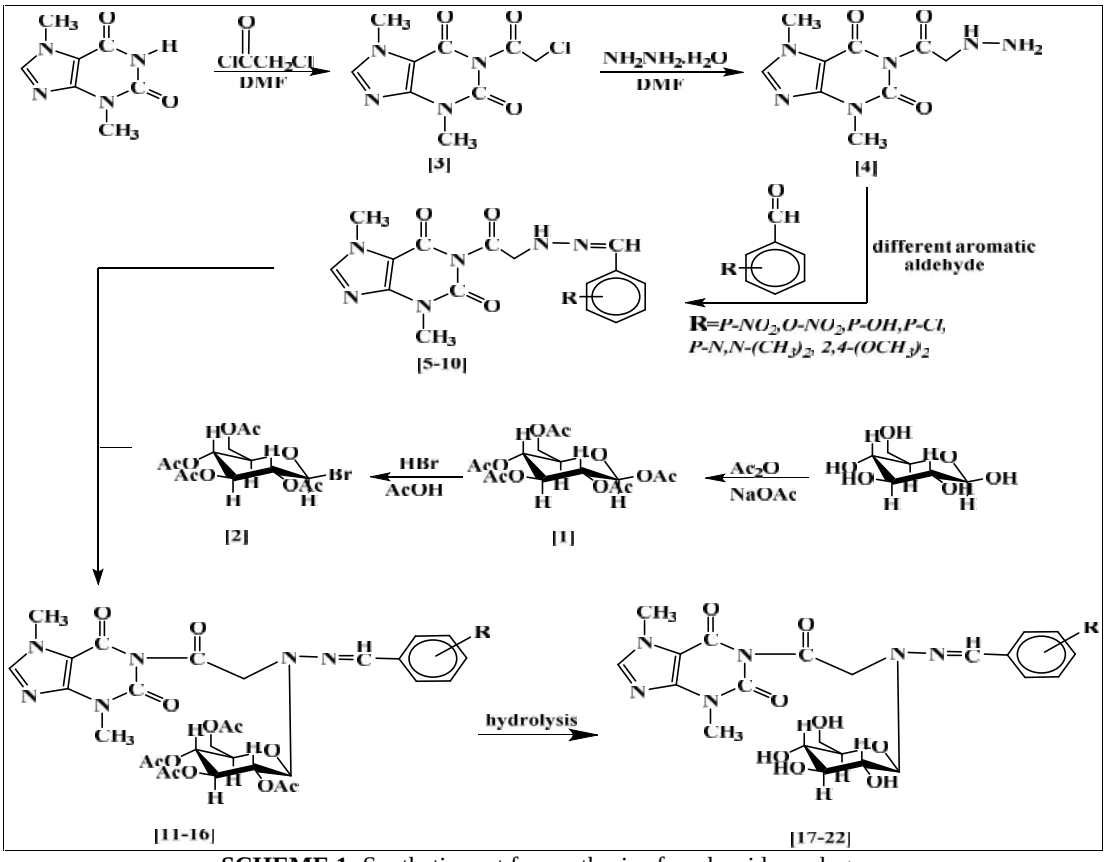
To a hot stirred solution of the hydrazide [4] (1 gm, 0.004 mol.) in DMF (10 ml.) appropriate aromatic aldehyde (0.004 mol.) was dissolved in ethanol containing 3-4 drops of glacial acetic acid. The reaction mixture was heated to (90-110)^{0}C for (4-6) hrs. The separated solid was put onto ice water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

General procedure for synthesis of protected nucleoside analogues [11-16] [35]

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. Tell the temperature raised to 137 ^{0}C, then cool (below 50 ^{0}C). The protected sugar [4,5] (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 and 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 give protected nucleoside analogues[11-16].

Hydrolysis of protected gluco- nucleoside analogues [17-22] (36)

A solution of (0.1g) of the protected nucleoside [11-16] in (10mL) of 0.1M methanolic sodium methoxide was refluxed for 0.5 hour TLC (DCM: EtOH 8:2) showed that the completion of reaction, then neutralized with acetic acid (5 drops) and evaporated to dryness, the residue was extracted with chloroform and the aqueous phase was removed to dryness in a vacuum, to give the nucleoside analogues [17-22] as shown in Scheme 1.



SCHEME 1- Synthetic rout for synthesis of nucleoside analogues

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 diameter 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. The obtained results are listed in table (1)

TABLE 1: Inhibition Zones of some newly synthesized compounds

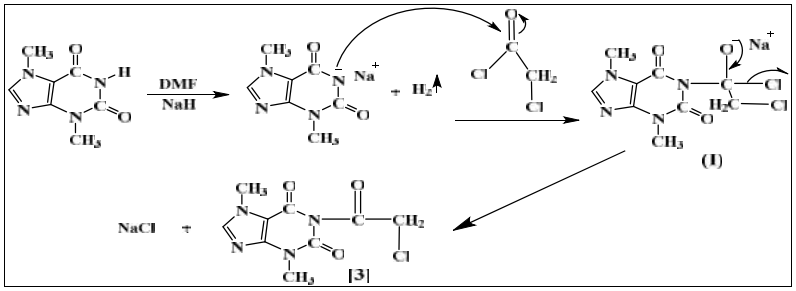
Comp. No.	Gram negative bacteria		Gram positive bacteria		Fungal
	E. coli	Pseudo.	Staph.	Strep	Asper.
Control	-	-	-	-	-
12	8	11	11	5	9
14	9	7	10	-	8
19	8	6	-	4	3
20	12	3	-	10	11
22	11	5	3	12	10

Solvent: DMSO (used as control) ; [C]=0.004 mole

Zone of inhibition: (-) no inhibition zone; (3-6) weak; (7-10) moderate; (11-16) strong.

RESULTS & DISCUSSION

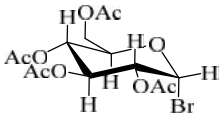
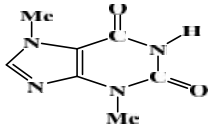
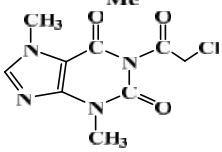
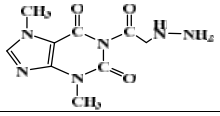
For the importance to synthesis new promising compounds act as antiviral or anticancer, We encouraged to synthesized new nucleoside analogues using theobromine as a nucleobase, (that is used for the first time in synthesis of nucleoside analogues field and Schiff base), and glucose used as a sugar moiety. The designed multisteps started with per acetylation of D. glucose using acetic anhydride and sodium acetate as a catalyst, then brominated [2] with HBr in glacial acetic acid. Theobromine was modified by conversion to it's 1-chloro acetyl derivative [3] via nucelophilic substitution of chloroacetyl chloride on the polarized N-H group of theobromine according to the suggested mechanism (scheme 2).



Scheme (2)

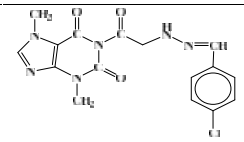
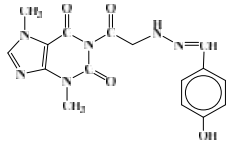
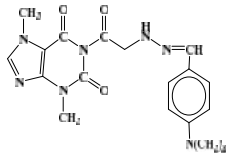
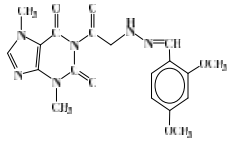
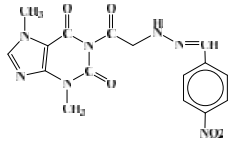
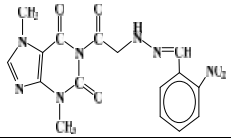
FTIR spectrum of compound [3] showed the appearance of absorption band at (1743) cm^{-1} due to ν (C=O) of acetyl group in addition of theobromine carbonyl [37]. The disappeared band of (NH) band gives a good evidence for formation of compound[3]. Compound[3] was reacted with hydrazine hydrate (95%) in DMF to give the key intermediate 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 [3,4] were listed in Table (2).

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
2		syrup	84.62	Color less	-	1736	-	633 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	ν(NH_2) (asy.3457) (sy.3396)

Compound [4] was treated with different substituted aromatic aldehydes to form the Schiff's bases of [5-10] (scheme1). The FTIR spectra of compounds [5-10] showed absorption bands at (3492-3309) cm^{-1} due to (NH) stretching. The ^{1}H-NMR spectrum of compound [6, 8] showed a singlet signal at δ= (8.8-10.09) ppm due to (N=CH) proton, a singlet signal at δ= (8.09-8.2) ppm due to (-NH) proton and disappearance of the signal of (NH_2) proton. spectral data are listed in the table (3 and 4).

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(N-H)	ν(C=O)	ν(C=N)	Others
5		155-160	96	Pale yellow	3309	1745 1691,1623	1591 1566	ν(C-Cl) 1087
6		199-203	71	Peggy	3323	1745 1683	1608 1581	ν(O-H) 3436
7		210	90	Reddish-orange	3492	1687	1602,1548	-
8		149-152	92	Pale yellow	3430	1708,1649 1612	1575 1553	ν(C-O-C) 1031,1269
9		172-173	94	Yellow	3407	1710 1699	1589	ν (NO_2) 1344,1519
10		198-194	85	Pale yellow	3479	1741,1677 1645	1629 1558	ν (NO_2) 1348,1527

To obtain our synthetic target the new protected nucleoside analogues following the Koenigs-Knörr condensation method by coupling of bromo acetylated glucose [2] with theobromine Schiff bases [5-10], through nucleophilic substitution afforded new blocked nucleoside analogues [11-16]. The FT-IR spectra of protected nucleoside analogues [11-16] showed the disappearance of υ (N-H) bands for hydrazone derivatives, and the appearance of stretching bands at (1747-1759) cm^{-1} for carbonyl group (acetyl for glucose) indicating successful coupling and the nucleoside analogues formation. The ^{1}H-NMR spectrum of compound [13] in δ ppm, showed a signal between (3.42-3.45) refer to ($H_6^{'}$, $H_6^{''}$). While multiplet signals between (3.57-5.63) due to other 5H for sugar protons. While the ^{13}C-NMR spectrum showed a signal between (70.15- 98.23) refers to sugar carbon. The signal at (173.21) for acetate (C=O) group, spectral data are listed in (table 5 and 6).

The acetylated nucleosides [11-16] were de blocked using methanolic sodium methoxide under reflux to afford our synthetic goal [17-22]. The FT-IR spectra of compounds [17-22] showed the disappearance of carbonyl band of ester group and the appearance of broad bands between (3207-3498) cm^{-1} for hydroxyl group of sugar moiety which give a good evidence for success and complete hydrolysis of nucleoside analogues. While the ^{13}C-NMR spectrum of compound [17] showed a signal between (69.81-86.61) refers to sugar carbon. Spectral data are listed in (table 7 and 8).

TABLE 4: ^{1}HNMR and ^{13}CNMR spectral data (δ ppm) of compounds [6,8]

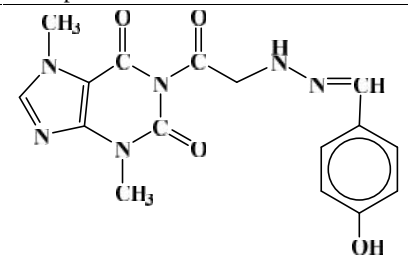
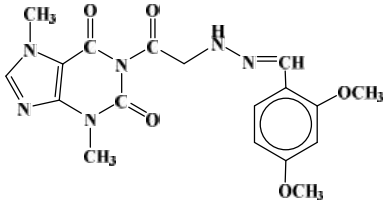
No.	Compounds	^{1}HNMR spectral data (δ ppm)	^{13}CNMR spectral data (δ ppm)
6		3.05(s,2H ,CH_2); 3.43 (s,6H , $2CH_3$); 6.8-7.9 (m,4H, aromatic) ; 8.09 (s,1H,NH) ; 10.09 (s,1H,CH=N benzylidine); 10.36(s,1H,CH imidazole); 11.26(s,1H,OH phenol).	30.01,34.9 (2C ,$2CH_3$); 43.88 (1C,CH_2); 115-147(6C , aromatic) ; 156.72 (1C, benzylidine); 159.167-160.355 (3C, imidazole); 161.37 (1C,C=O amide); 164.42, 164.55(2C,C=O theo).
8		3.35(s,3H,OCH_3); 3.80-3.91(9H,OCH_3 and $2NCH_3$); 3.98(s,2H ,CH_2); 6.58-7.75(m,3H , aromatic); 8.2(s,1H,NH); 8.82(s,1H,CH=N benzylidine); 11.32(s,1H,CH imidazole).	55.37-55.82(4C,$4CH_3$); 98.1(1C,CH_2); 106.2-127.6(6C, aromatic); 143.0(1C,benzylic); 156.4-160.1(3C,imidazole); 162.20(1C,C=O amide); 163.33,164.53(2C,C=O Theo).

TABLE 5: the physical properties and the Fourier infrared values

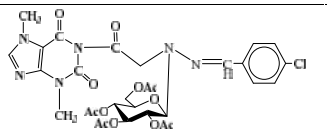
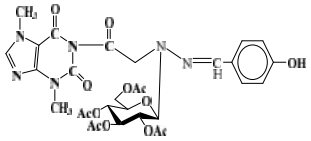
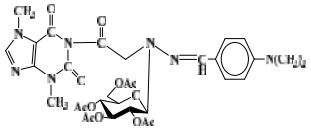
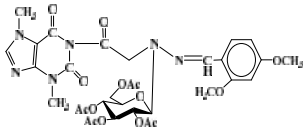
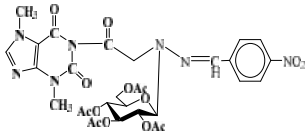
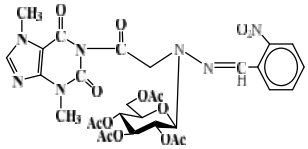
Comp. NO.	Physical properties				FTIR absorption cm^{-1}			
	Comp. structure	Melting point °C	Yield %	Color	ν (C-H) aromatic	ν(C=O)	ν(C=N)	Others
11		172- 175	79	Yellowish – orange	3020	1749, 1725 1693, 1623	1591 1564	ν(C-Cl) 1170
12		Syrup	59	Yellow	3029	1753, 1725 1691	1599 1530	ν(O-H) 3442
13		Syrup	73	Reddish-orange	3033	1749, 1694, 1645	1608 1539	-
14		Syrup	55	Yellow	3025	1749, 1710, 1668	1582 1561	ν(C-O-C) 1253,1029
15		185-188	54	Yellow	3083	1751, 1714, 1650	1595	ν (NO_2) 1346,1523
16		250-254	51	Deep yellow	3043	1749, 1701, 1652	1623 1563	ν (NO_2) 1339,1530

TABLE 6: ^{1}HNMR and ^{13}CNMR spectral data (δ ppm) of compound [13]

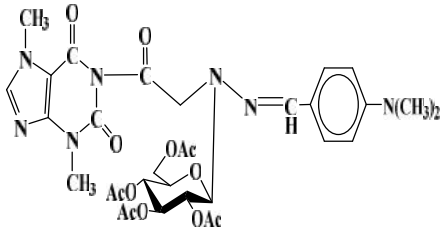
No.	Compound	^{1}HNMR spectral data (δ ppm)	^{13}CNMR spectral data (δ ppm)
13		1.82-2.00(s,12H,4CH_3 acetyl); 2.9(s,6H, 2 NCH_3); 3.039 (s,6H,2 NCH_3 Theo); 3.40 (s, 2H,CH_2); 3.42-3.45(2H,H'_6 , H''_6); 3.57-5.63 (m,5H,sugar protons); 6.76-7.54(m, 4H, aromatic); 9.50 (s,1H, CH=N benzylidine) ; 9.61(s,1H,C-H imidazole).	20.72(4C,CH_3-C=O); 31.8,32.5 (2C, 2CH_3Theo);41.86(2C,N-CH_3); 60.03 (1C,CH_2); 70.15-98.23(6C,sugar); 111.132-133.3(6C, aromatic); 142.74 (1C,benzylic); 152.4, 169.72, 169.99 (3C,C=O theo&amide); 173.21(4C,CH3-C=O).

TABLE 7: the physical properties and the Fourier infrared values

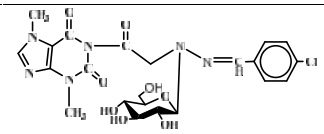
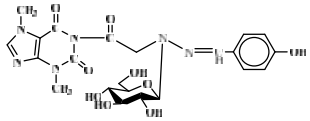
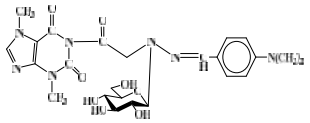
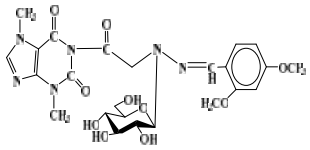
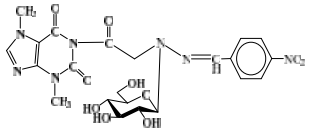
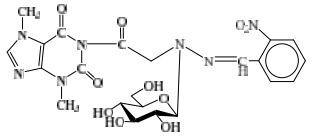
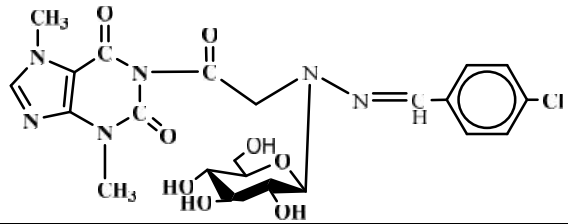
Comp. NO.	Physical properties				FTIR absorption cm^{-1}			
	Comp. structure	Melting point °C	Yield %	Color	ν (O-H)	ν(C=O)	ν(C=N)	Others
17		150- 154	92	Yellow	3260-3477	1645	1562	ν (C-Cl) 1168
18		Syrup	78	Pale yellow	3365, 3525	1650, 1668, 1697	1560	-
19		96- 98	78	Reddish-brown	3309-3396	1649	1562	-
20		Syrup	81	Pale yellow	3350-3481	1641	1573	ν (C-O-C) 1049,1256
21		Dec. 175	92	Pale yellow	3392, 3440	1641, 1678	1571	ν (NO_2) sy. 1342, asy.1562
22		Dec. 209	83	Yellow	3327, 3451	1654	1584	ν (NO_2) sy. 1349, asy.1538

TABLE 8: ^{13}C–NMR – Spectral data for compound [17]

No.	Compounds	δ ppm
17		25.02,38.6(6C,2CH_3); 60.66(1C, CH_2); 69.81-86.61(6C,sugar); 114-125(6C, aromatic); 144.6(1C,benzylic) ; 144.61-146.8(3C,imidazole); 151.38(1C,C=O amide);175.12,175.42(2C,C=O theo).

CONCLUSION

This work demonstrates the reactions involved in the synthesis of new nucleoside analogues and hence, the detection of their antimicrobial activity. The antimicrobial activity of these compounds was estimated against Gram-positive, Gram-negative bacteria and fungi. On the other pointer, most of the compounds exhibited a moderately significant antimicrobial activity.

REFERENCES

[1]. Palmu, K., Rosenqvist, P., Thapa, K., Ilina, Y., Siitonen, V., Baral, B., & Metsä-Ketelä, M. (2017) Discovery of the Showdomycin Gene Cluster from Streptomyces Showdoensis ATCC 15227 Yields Insight into the Biosynthetic Logic of C-Nucleoside Antibiotics. *ACS Chemical Biology*.

[2]. Clercq, E. D. (2013) Highlights in antiviral drug research: antivirals at the horizon. *Med. Res. Rev*. **33**, 1215–1248.

[3]. Shelton, J., Lu, X., Hollenbaugh, J. A., Cho, J. H., Amblard, F., Schinazi, R. F. (2016),Metabolism, Biochemical Actions, and Chemical Synthesis of Anticancer Nucleosides, Nucleotides, and Base Analogs. *Chem. Rev*., 116 (**23**), 14379–14455.

[4]. Crespan, E., Garbelli, A., Amoroso, A., Maga, G. (2011), Exploiting the Nucleotide Substrate Specificity of Repair DNA Polymerases to Develop Novel Anticancer Agents. *Molecules*, **16**, 7994–8019.

[5]. Vitali, L.A., Petrelli, D., Lambertucci, C., Prenna, M., Volpini, R., Cristalli, G. (2012) In vitro antibacterial activity of different adenosine analogues. *J. Med. Microbiol*., **61**, 525–528.

[6]. Xavier, N.M., Schwarz, S., Vaz, P.D., Csuk, R., Rauter, A.P.(2014), Synthesis of Purine Nucleosides from D-Glucuronic Acid Derivatives and Evaluation of Their Cholinesterase-Inhibitory Activities. *Eur. J. Org. Chem*., **2014**, 2770–2779.

[7]. Schwarz, S., Csuk, R., Rauter, A.P. (2014), Microwave-assisted synthesis of novel purine nucleosides as selective cholinesterase inhibitors. *Org. Biomol. Chem*., **12**, 2446–2456.

[8]. Batista, D., Schwarz, S., Loesche, A., Csuk, R., Costa, P.J., Oliveira, M.C., Xavier, N.M. (2016),Synthesis of glucopyranos-6′ -yl purine and pyrimidine isonucleosides as potential cholinesterase inhibitors. Access to pyrimidine-linked pseudo-disaccharides through Mitsunobu reaction. *Pure Appl. Chem*., **88**, 363–379.

[9]. Xia, B. W., Zhang, Y. C., Wang, J., Ding, F. H., & He, X. D. (2015). Efficacy of antiviral therapy with nucleotide/nucleoside analogs after curative treatment for patients with hepatitis B virus-related hepatocellular carcinoma: a systematic review and meta-analysis. *Clinics and research in hepatology and gastroenterology*, *39*(**4**), 458-468.

[10]. Zhang, J., Ying, H., Wei, L., & Hong, L. J. (2017) Effect of nucleoside analogues in the treatment of hepatitis B cirrhosis and its effect on Th17 cells. *European review for medical and pharmacological sciences*, *21* (**2**), 416-420.

[11]. Kasraianfard, A., Watt, K. D., Lindberg, L., Alexopoulos, S., & Rezaei, N. (2016). HBIG remains significant in the era of new potent nucleoside analogues for prophylaxis against hepatitis B recurrence after liver transplantation. *International reviews of immunology*, *35*(**4**), 312-324.

[12]. Yuen MF, Lai CL. (2011), Treatment of chronic hepatitis B: evolution over two decades. *J Gastroenterol Hepatol*, 26 Suppl **1**: 138–43.

[13]. Terrault NA, Bzowej NH, Chang KM, Hwang JP, Jonas MM, Murad MH, (2016),American Association for the Study of Liver Diseases. AASLD guidelines for treatment of chronic hepatitis B. *Hepatology*; **63**:261–83.

[14]. Ly KN, Hughes EM, Jiles RB, Holmberg SD. 2016. Rising mortality associated with hepatitis C virus in the United States, 2003–2013. Clin. Infect. Dis. **62**:1287–88

[15]. Naggie, S., & Muir, A. J. (2017). Oral combination therapies for hepatitis C virus infection: successes, challenges, and unmet needs. *Annual review of medicine*, ***68***, 345-358.

[16]. Konerman MA, Mehta SH and Sutcliffe CG 2014. Fibrosis progression in human immunodefi- ciency virus/hepatitis C virus coinfected adults: prospective analysis of 435 liver biopsy pairs. *Hepatology* 59(**3**):767–75

[17]. Rockstroh JK, Nelson M, and Katlama C 2015. Efficacy and safety of grazoprevir (MK-5172) and elbasvir (MK-8742) in patients with hepatitis C virus and HIV co-infection (C-EDGE CO-INFECTION): a nonrandomised, open-label trial. *Lancet HIV* 2(**8**):e319–27

[18]. Fyfe, J., Keller, P., Furman, P., Miller, R., Elion, G. (1978), Thymidine kinase from herpes simplex virus phosphorylates the new antiviral compound, 9-(2-hydroxyethoxymethyl) guanine. *J. Biol. Chem*., **253**, 8721–8727.

[19]. Smith, K.O., Galloway, K.S., Kennell, W.L., Ogilvie, K.K., Radatus, B.K. (1982), A new nucleoside analog, 9-[[2-hydroxy-1-(hydroxymethyl) ethoxyl] methyl] guanine, highly active in vitro against herpes simplex virus types 1 and 2. *Antimicrob. Agents Chemother*., **22**, 55–61.

[20]. Vince, R., Hua, M., Brownell, J., Daluge, S., Lee, F., Shannon, W.M., Lavelle, G.C., Qualls, J., Weislow, O.S., Kiser, R. (1988), Potent and selective activity of a new carbocyclic nucleoside analog (carbovir: NSC 614846) against human immunodeficiency virus in vitro. *Biochem. Biophys. Res. Commun.*, **156**, 1046–1053.

[21]. Rabkin, C. (2001), AIDS and cancer in the era of highly active antiretroviral therapy (HAART). *Eur. J. Cancer*, **37**, 1316–1319.

[22]. Mallal, S.A., John, M., Moore, C.B., James, I.R., McKinnon, E.J. (2000) Contribution of nucleoside analogue reverse transcriptase inhibitors to subcutaneous fat wasting in patients with HIV infection. *Aids*, **14**, 1309–1316.

[23]. Bilkan, M. T. (2017) Structural and spectroscopic studies on dimerization and solvent-ligand

complexes of Theobromine. *Journal of Molecular Liquids*, ***238***, 523-532.

[24]. Kerimi, A., & Williamson, G. (2015). The cardiovascular benefits of dark chocolate. *Vascular pharmacology*, ***71***, 11-15.

[25]. González-Calderón, D., González-Romero, C., González-González, C. A., & Fuentes-Benítes, A. (2015). Synthesis of caffeine from theobromine: bringing back an old experiment in a new setting. *Educación Química*, *26*(**1**), 9-12.

[26]. Brodowska, K., & Lodyga-Chruscinska, E. (2015). Schiff bases—interesting range of applications in various fields of science. *ChemInform*, *46*(11).

[27]. Kamaria, P., Kawathekar, N., & Chaturvedi, P. (2011). Microwave assisted synthesis and antimicrobial evaluation of Schiff bases of indole-3-aldehyde. *Journal of Chemistry*, *8*(**1**), 305-311.

[28]. Bringmann, G., Dreyer, M., Faber, J. H., Dalsgaard, P. W., Stærk, D., Jaroszewski, J. W., & Christensen, S. B. (2004). Ancistrotanzanine C and Related 5, 1 '- and 7, 3 '-Coupled Naphthylisoquinoline Alkaloids from Ancistrocladus t anzaniensis 1. *Journal of natural products*, *67*(**5**), 743-748.

[29]. Guo, Z., Xing, R., Liu, S., Zhong, Z., Ji, X., Wang, L., & Li, P. (2007). Antifungal properties of Schiff bases of chitosan, N-substituted chitosan and quaternized chitosan. *Carbohydrate Research*, *342***(10)**, 1329-1332.

[30]. Vogel A., (**1971**), "Atext Book of Paractjical Organic Chemistry" Logman Group Limited. P.452

[31]. Tipson , S.R.In , (**1963**). Methods in carbohydrate chemistry , Vol.(II), (Wistter ,R.L. and Wolfrom , M.L., eds) , p.247 , A cademic press Inc. New York and London. (a) p.247 , (b) p.319, (c) p.226

[32]. Abu Mohsen U. (2014)," studies on benzimidazole dithiocarbamate derivatives as acetycholinesterase inhibitors" Faculty of Pharmacy Department of Pharmecutical Chemistry, Al-Azhar University, Gaza,Palestine. Vol 3, Issue 12, p.11

[33]. Sammaiah.G and Pragathi. K,(2014) "synthesis and evaluation of new bis isatin derivatives for antioxidant and cytotoxic activities" University College of Pharmaceutical Sciences, Kakatiya University, Warangal. Vol 3, Issue 7,p.557

[34]. Al-Majidi, S. (2013) " Synthesis, Characterization and Evaluation of Antimicrobial Activity of Several New N-Substituted Carbazole" Department of Chemistry, College of Science, University of Baghdad, Baghdad-Iraq. Vol.16 (4), December, p.69

[35]. Al-Mouman T. (2007), "Synthesis of 2,3 dideoxy 3,3 gem-di-C- alkyl pyrono nucleouside analogues" Ph.D thesis department of chemistry college of science university of Bagdad, Iraq.

[36]. Zemplen G., Gerecs A. and Hadacsy, (1936), I, Ber, **69**, 1827.

[37]. Robert, M. S., Francis, X. W. and David, J. K. (2005) (spectrometric identification of organic compounds) 7th ed., john wiley & sones, USA.