ISSN: 0067-2904
GIF: 0.851

Synthesis and Characterization of Some New Nucleoside Analogues from Substituted Benzimidazole and Evaluation of Their Biological Activities

Thanaa M. Al-Mouamin[1*], Ahmed Kh. Kadhim[2]
[1]Department of Chemistry, College of Science, Baghdad University, Baghdad, Iraq.
[2] Ministry of Environment, Baghdad, Iraq.

Abstract

This research includes the synthesis of some new nucleoside analogues via 1,3-dipolar cycloadditon reaction starting with 1,3,4,6- Tetra- O-benzoyl –2 – azido-2-deoxy- β – D- fructofuranose (F3) which was prepared in three steps ,protection (benzoylation) , bromination and azotation ((F1-F3) from D- fructose as sugar moiety while the base moiety , 2-substituted benzimidaole derivatives (A1-A5) were synthesized by condensation of O-phenylenediamine with different aromatic aldehyde .

Nucleophilic substitution of propargyl bromide with benzimidaole derivatives (gave the N- substituted compounds (B1-B5) which were reacted with azido sugar through 1,3- dipolar cycloadditon reaction to give nucleoside analogues (C1-C5) . Debloking of these nucleoside using sodium methoxide in methanol afforded our target the free nucleoside analogues (D1-D5).

All prepared compound were identified by FT-IR and some of them with ^{1}H-NMR and ^{13}C-NMR. The synthesized compounds (D1-D5) were screened for their in-vitro antibacterial and antifungal activity.

Keywords: Nucleoside analogues, Benzimidazole, Trizole, Antibacterial

تحضير وتقدير لمماثلات نيكليوسيدات جديدة محضرة من بنزايميدازول المعوض وتقييم الفعالية البايولوجية

ثناء مهدي المؤمن[١*] ، أحمد خضير كاظم[٢]
[١]قسم الكيمياء ، كليةالعلوم ، جامعة بغداد، بغداد، العراق
[٢]وزارة البيئة ، دائرة بيئة الوسط، بغداد ، العراق

الخلاصة

يتضمن البحث تحضير بعض مماثلات النيوكليوسيدات الجديدة بوساطة الاضافة ١,٣- ثنائية القطب الحلقية بدءاً من سكر ١,٣,٤,٦-رباعي –O- بنزويل -٢- ازيدو -٢- ديوكسي –β–D- فركتوفيورانوز (F3) والذي حضر بثلاث خطوات من D- فركتوز ، حماية بالبنزويل والتعويض بالبروم ثم بالازايد (F1- F3) .

اما جزء القاعدة تم تحضيرها من تكاثف O–ثنائي فنيلين امين مع الديهايدات أروماتية مختلفة أعطى مشتقات البنزاميدازول المعوض في الموقع (٢) (A1–A5) ان التعويض النيوكليوفيلي لبروميد البروبارجيل مع (A1–A5) أعطى مركبات معوضة على النايتروجين في الموقع ١ (B1–B5) والتي تم تكثيفها من خلال الاضافة ١,٣- ثنائية القطب الحلقية مع سكر الازايد (F3) لتعطي مماثلات النيوكليوسيدات المحمية (C1–C5) والتي بعد تحللها القاعدي بايون الميثوكسيد اعطت النيوكليوسيدات الحرة (D1–D5). تم تشخيص المركبات و النيكليوسيدات المحضرة بواسطة طيف الاشعة تحت الحمراء والبعض منها بطيف الرنين النووي

*Email:thanaa_ma2007@yahoo.com

المغناطيسي للبروتون ^{1}H–NMR و نظير الكربون ١٣ ^{13}C–NMR. كما تم دراسة الفعالية البايولوجية للمركبات (D1–D5) خارج الخلية لانواع من البكتريا ونوع من الفطر.

Introduction

Nucleoside analogues have been in clinical use for almost 50 years and have become cornerstones of treatment for patients with cancer or viral infections [1].

The nucleoside analogues are an important class of antiviral agents now commonly used in the therapy of human immunodeficiency virus (HIV) infection, hepatitis B virus (HBV), cytomegalovirus (CMV) and herpes simplex virus (HSV) infection [2]. Nucleoside analogues are a large class of agents that include drugs for cancer (cytarabine, gemcitabine, mercaptopurine), rheumatologic diseases (azathioprine, allpurinol) and even bacterial infections (trimethoprim) [3].

Benzimidazole and its dervatives are one of the most promising heteroaryl moieties that yielded many successful drugs [4] dervatives of these compounds are known for their antibacterial and antifungal activities [5]. The success with these compounds stimulated the search for new biologically active dervatives. Some of these compounds exhibited anticancer [6], antiviral [7], anti-inflammatory [8], antihypertensive [9] and anticoagulant agents [10]. On the other hand also trizole and its derivatives have abiological activites and antifungal that made several drugs such as fluconazole, isavuconazole, itraconazole, voriconazole, pramiconazole, ravuconazole, and posaconazole [11]. Considering the above significances our research concern with synthesis of new nucleoside analogues containing 2-substuted benimidazole and trizole.

Experimental

Instruments

- Melting points were recorded by **Gallen Kamp**, England .melting point apparatus and were uncorrected.
- Infrared spectra were recorded using Fourier Transform infrared **SHIMADZU** (8300) (FTIR) infrared spectrometer, Japan,as KBr disc or thin film .
- ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Burker, Ultra shield 300MHz, Jordan, Amman, using tetramethyl silane as internal standard and DMSO-d_6 as a solvent.
- Biological activity using incubator Memmert.
- The DMSO-d_6 solvent appeared at 2.5ppm in ^{1}H-NMR and at 40.45ppm in ^{13}C-NMR spectrum[12]

Chemicals

- All chemical starting compounds were obtained from Fluka , Aldrich and BDH

General procedure

Synthesis of 1,3,4,6- tetra –O-benzoyl –β-D- fructofuranose [13]

Benzoyl chloride (7 ml) was added dropwise to anhydrous –D- fructose (2 g, 11.11mmol) that suspended in mixture of chloroform (30 ml) and dry pyridine (5ml), Then the mixture was heated on water bath at (45-65)˚C with continuous stirring for 4 h. the end of reaction was indicated by TLC ($CHCl_3$:MeOH ; 8:2) The mixture was poured over ice –water then extracted with $CHCl_3$ (3×15ml). The organic phase was washed with (10 ml) (5% HCl) solution (to remove unreacted pyridine), and the organic phase was neutralized with (5% Na_2CO_3) solution (10ml) , the organic phase dried over anhydrous sodium sulphate and the solvent evaporated to give a syrup that crystallized from absolute ethanol to give white crystals (m.p. 121- 122˚C)

Synthesis of 1, 3, 4, 6- Tetra- O-benzoyl – β – D- fructofuranosyl bromide

Hydrogen bromide in glacial acetic acid (5 ml) of (45%) was added to tetrabenzoyl fructofuranose (2g, 3.36 mmol) then (5 ml) of glacial acetic acid was added . the mixture was stirred for 30 min.(room temperature) and left for 6 h. at room temperature, then the mixture left over night at (5 ˚C) the end of reaction was checked by TLC [$CHCl_3$: MeOH; 8:2]) after that mixture was neutralized with saturated aqueous sodium bicarbonate solution and extracted with chloroform (3× 15ml) , dried over anhydrous sodium sulphate , filtered , evaporated in vacuo to give a brown syrup .

Synthesis of 1,3,4,6- Tetra- O-benzoyl –2 – azido-2- deoxy- β – D- fructofuranose

Excess of sodium azide was added to (1, 3, 4, 6- Tetra- O-benzoyl – β – D- fructofuranosyl bromide) in 20 ml DMF, the mixture was heated with stirring (50- 60) ˚C for 20 h. The end of reaction was checked by TLC (benzene: MeOH; 8:2).

The mixture was poured into ice-cold water and extracted with chloroform (3× 15ml) , dried with anhydrous sodium sulphate , filtered , evaporated in vacuo to give the product as a brown syrup .

General procedure for the synthesis of 2- substituted phenyl benzimidazole [14]

(0.039g, 0.3 mmol) of $NaHSO_3$ was added to amixture of (1.08g , 10 mmol) of O-phenylene diamine and (10 mmol) of substituted aromatic aldehyde in (5 ml) of DMF then heated with stirring at 80˚C for 4h., distilled water (20 ml) was added to the above mixture and then filtered , recrystallization was achieved by using 30%ml ethanol.

General procedure for the preparation of 1-propynyl – 2-substituted phenyl benzimidazole

Prepared benzimidazole derivative (5 mmol) was heated under refluxe with alcoholic potassium hydroxide (4 M) for 0.5 h. then (0.44ml, 5 mmol) of propargyl bromide was added and was heated under reflux in boiling water bath for 3-4 h. , filtered and recrystalizied from ethyl acetate.

General procedure for the preparation of nucleoside analogues [15]

(10 mmol) 1-propynyl – 2-substiuted phenyl benzimidazole was added to 1,3,4,6- Tetra- O-benzoyl –2 – azido-2- deoxy- β – D- fructofuranose (10 mmol) with Cu^{I}(0.008mmol) as acatalyst in presence of base then sterred for 72 h. at room temperature.

Hydrolysis of nucleoside analogues

A solution of (0.15 g) of the blocked nucleoside in (7 ml) of (0.1 M) methanolic sodium methoxide was refluxed with stirring for 0.5 h. The mixture was neutralized with acetic acid and evaporated to dryness. The residue was partitioned between water and chloroform and the aqueous phase evaporated to dryness in vacuo. The residue was recrystallized from ethanol ether.

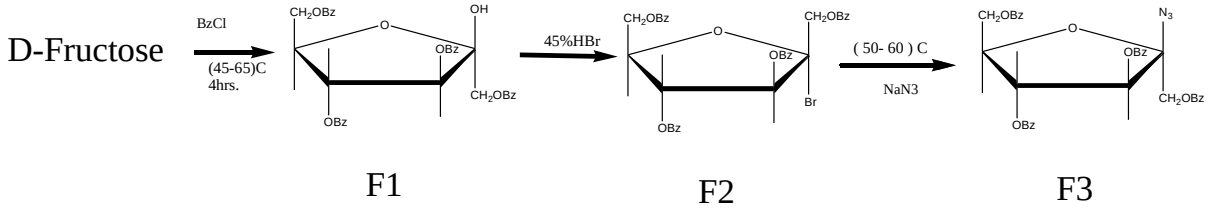
Anti-microbial activity test

The test was performed according to the disk diffusion method. Some of prepared compounds were tested against two strain gram +ve (*staph aureas* and *stphylo coccus*) and two strain gram –ve bacteria (*E.coli* and *proteus vulgaris*). Also they tested against one strain of yeast (***Candida***). Whattman no.1 filter paper disk of 5mm diameter were sterilized by autoclaving for 15 min. at 121 ◦C. Agar plates were surface inoculated uniformly with 100 ◦ µL from both culture of tested microorganism. The impregnated disk were placed on the medium suitably spaced a part and the plates incubated at 5 ◦C for 1 hr. to permit good diffusion and then transferred to an incubator at 37 ◦C for 24 hrs. . The inhibition zones caused by various compounds on the microorganisms were examined.

Result and discussion

Structurally modified nucleosides represent an important class of medicinal compounds which have been found to behave as therapeutic agents and are currently used in pharmaceuticals as antitumor, antiviral and antibiotic agent [16].

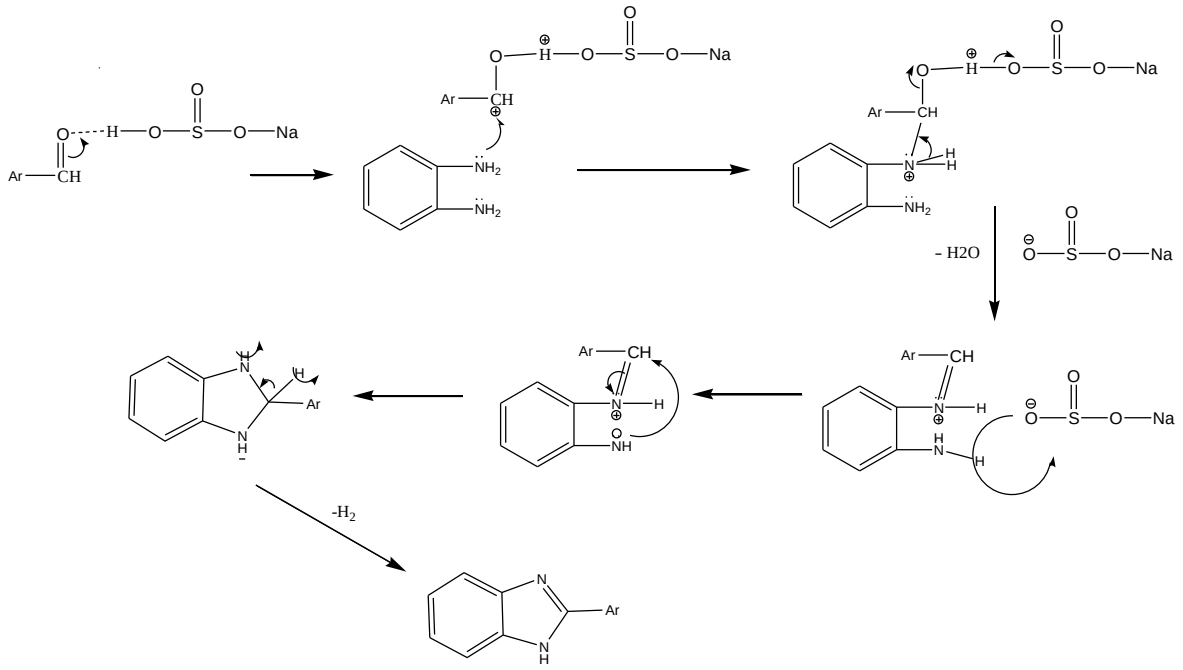
Therefore our strategy for design nucleoside analogues involving modification of nucleobase and sugar moiety. D-Fructose chosen as starting material which was protectied with benzoyl chloride leading the tetra–*O*-benzoyl –*β-D*- fructofuranose leaving hydroxyl group at C_2 for ferther reaction, which was brominated using hydrogen bromide in glacial acetic acid, then trated with sodium azide to obtain our synthone the azido sugar which was subjected to 1, 3-dipolar cycloaddition Scheme-1.



Scheme 1-synthesis of sugar moiety

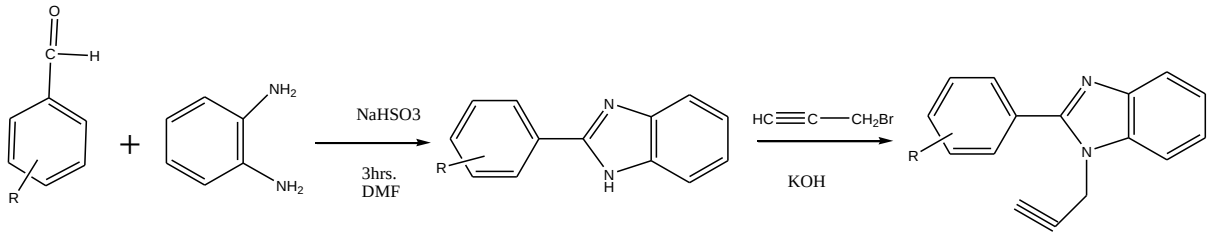
These compounds (F1-F3) confirmed by their physical properties that given as literature [13] and FT-IR spectrum. The spectrums showed several characteristic bands mainly the carbonyl group of benzoyl appeared at (1720 cm^{-1}) while, F2 showed the disappearance of hydroxyl band and appearance of C-Br at(686 cm^{-1}). The azido sugar (F3) showed disappearance of C-Br band and a stretching band of N_3 at (2123cm^{-1})

On the other hand, benzimidazole is an important pharmacophore due to the structural similarty of purine [15]. There for was chosen as a nucleobase , it was synthesized by condensation of *O*-phenylene diamine with different aromatic aldehyde using sodium hydrogen sulfite as ring closing agent , according mechanism showed in Scheme-2.



Scheme 2-Mechanism for the synthesis of benzimidazole

For ferther modification of nucleobase , substituted benzimidazole (A1-A5) were undergoing nucleophilic substitution with propargyl bromide to give 1-proynyl -2- substituted benzimidazole derivatives (B1-B5) Scheme-3.



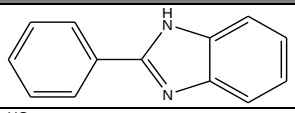
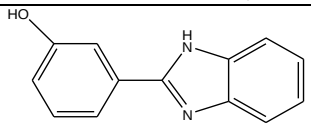
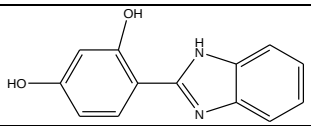
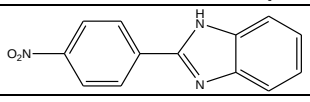
A1-A5 B1-B5

R=-H; 3-0H; 2, 4-OH; 4-NO_2 ; 4-$N(CH_3)_2$

Scheme 3-Synthesis of compounds (A1-A5) (B1-B5)

Physical properties of the prepared compounds and other data are listed in Table-(1and2)

Table 1- Physical properties of compounds (A1-A5)

Comp. No.	Compound Structure	Molecular formula	M.wt (g/mol)	m.p°C	Color
A1		$C_{13}H_{10}N_2$	194	211-214	orange
A2		$C_{13}H_{10}N_2O$	210	282-285	orange
A3		$C_{13}H_{10}N_2O_2$	226	296-300	red
A4		$C_{13}H_9N_3O_2$	239	311--315	yellow

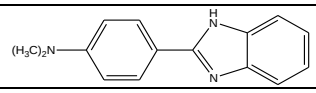
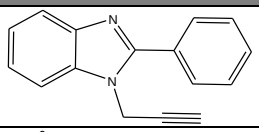
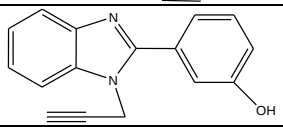
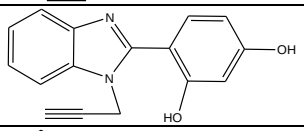
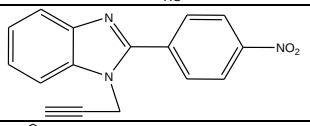
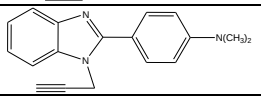
A5		$C_{15}H_{15}N_3$	237	305--307	yellow

Table 2- Physical properties of compounds (B1-B5)

Comp. No.	Compound Structure	Molecular formula	M.wt (g/mol)	m.p	Color
B1		$C_{16}H_{12}N_2$	232	Oil	brown
B2		$C_{16}H_{12}N_2O$	248	Oil	brown
B3		$C_{16}H_{12}N_2O_2$	264	320-322	Yellow
B4		$C_{16}H_{11}N_3O_2$	277	296-300	yellow
B5		$C_{18}H_{15}N_3$	273	288-290	yellow

All these compounds were identified by FT-IR spectroscopy there characteristic bands listed in Table-3.

Table 3- FT-IR spectral data $cm^{-1}$for compounds (A1-A5, B1-B5, C1-C5and D1-D5)

Comp.	ν(N-H)	**ν(C-H) aromatic**	**ν(C-H) aliph.**	**ν(C=C) aromatic**	ν(C=N)	**ν(C=O)**	ν(O-H)	other
A1	3360	3056	--	1583	1616	-	-	
A2	3440	3055	-	1578	1660	-	3280	
A3	3371	3029	-	1529	1685	-	3249	
A4	3425	3078	-	1523	1608	-		1523 (NO_2)
A5	3568	3040	2855	1565	1645	-		
B1	-	3055	2890	1578	1680	-		
B2	-	3058	2850	1589	1656	-	3267	2185 (**C≡C**)
B3	-	3045	2871	1576	1600	-	3201	2102(**C≡C**)
B4	-	3040	2875	1560	1620	-	-	1523 (NO_2) 2100(**C≡C**)
B5	-	3055	2890	1548	1598	-	-	2129(**C≡C**)
C1	--	3048	2912	1590	1618	1720		1600(N=N)
C2	-	3050	2823	1580	1600	1726	3425	1600(N=N)
C3	-	3038	2890	1588	1602	1716		1600(N=N)
C4	-	3040	2889	1576	1595	1723	-	1600(N=N) 1515(NO_2)
C5	-	3056	2880	1598	1634	1740	-	1600(N=N)
D1	-	3058	2920	1587	1604	-		1600(N=N)
D2	-	3044	2918	1567	1600	-	3444	1600(N=N)
D3	-	3052	2878	1556	1616	-	٣٤٠٠	1603(N=N)
D4	-	3055	2814	1580	1603	-	3450	1620(N=N) 1520(NO_2)
D5	-	3050	2844	1587	1622		3420	1600(N=N)

The FT_IR of benzimidazole compounds (A1-A5) showed a stretching band at 3360 cm^{-1} for primary amine, while (A2)and (A3) showed, in additional to the amine group, a stretching bands at 3280 cm^{-1} and 3249 cm^{-1} respectively for phenolic OH. (A4) compound showed a stretching band at 1523 cm^{-1} for NO_2 group. Compounds (B1-B5) showed in addition of the above, an acetylenic stretching bands between (2100-2185cm^{-1}) and disappearance of the primary amine band. Which was demonstrated substitution of propynyl on nitrogen Figure-1.

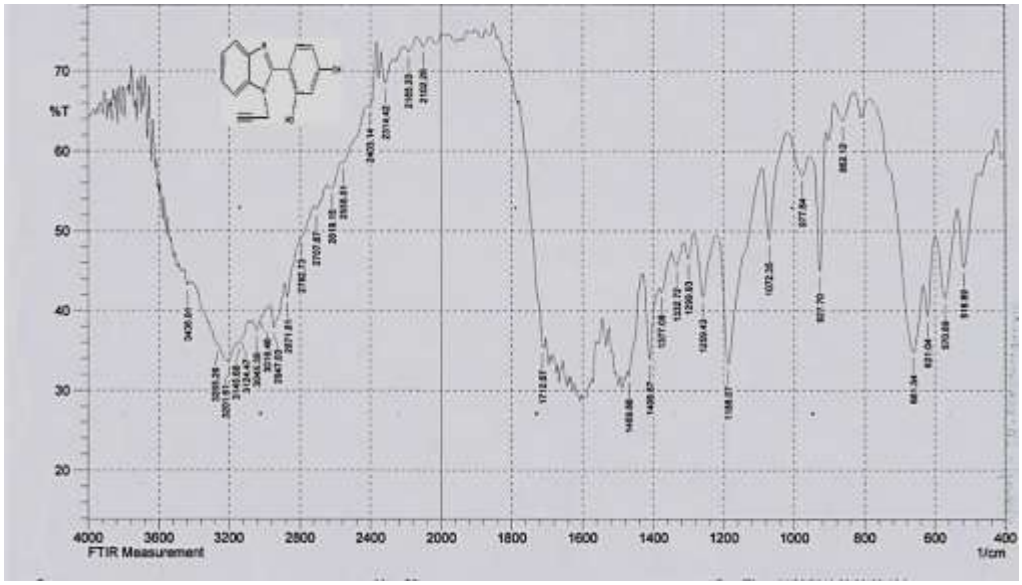
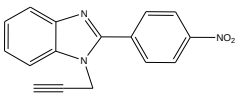
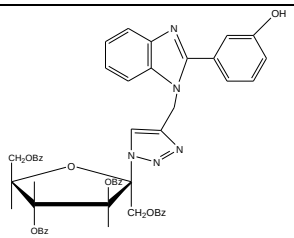
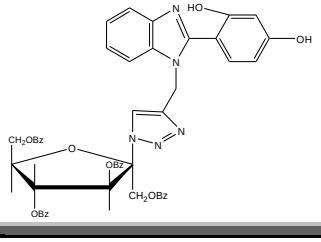


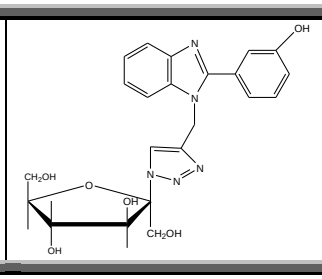
Figure1- FT-IR Spectrum for compound B3

Compound (B4) confirmed also by ^{1}H-NMR and ^{13}C_NMR spectroscopy , the ^{1}H-NMR spectrum (Table- 4)showed two singlet signals 2.97 and 3.327 ppm for CH_2 and terminal acetylenic proton respectively , aromatic protons appeared as multiplet at 6.64-8.1 ppm.

The ^{13}C_NMR spectrum Figure-2, Table-5 showed signal at 38.73 ppm for probergyl CH_2, while the carbon triple bond appeared at 64.3 ppm and 72.45 ppm. The aromatic singals appeared at 116.88-135 ppm while the imidazole carbon appeared at low field region at 159.19 ppm.

Table 4- ^{1}H-NMR spectral data for compounds (B4, C2, C3 and D2)

Comp. No.	Structures	^{1}HNMR Spectral data(δ ppm)
B4		2.97 (s,2H,CH_2).; 3.327(S,1H, actylenic) 6.64 -8.10 (m,8H,Ar-H)
C2		2.4(s,4H,2CH_2 Sugar protons, H′$_1$, H″$_1$,H′$_6$ and H″$_6$); 3.3(s,2H, methylene protons) ; 4.41-5.66(m,3H,sugar protons H_3,$H_4$and H_5);6.37-7.94(m,28H,Ar-H) ; 9.69(s,1H,OH phenolic)
C3		2.2(s,4H,CH_2 Sugar protons,H′$_1$, H ″$_2$, H′$_6$ and H ″$_6$) 3.06 (s,2H,CH2Methylene ,) 3.73-3.99,4.25-4.49 and 5.49-5.6(3H ,sugar protons ,H_3,H_4.H_5) ; 9.93 (S,2H ,2OH Phenolic)

D2		2.49(s,4H,2CH_2 sugar protons , H′$_1$,H″$_1$, H′$_6$ and H″$_6$); 2.656 (s, 2H , methylene protons) ; 3.344(s , 4H , 4OH) ; 3.5-3.6 , 4.1 -4.67 and 5.23 -5.303 (3H , sugar protons H_3 , H_4 and H_5); 6.15-7.69 (m, 8H, Ar-H) ; 9.29 (s , 1H , OH phenolic .

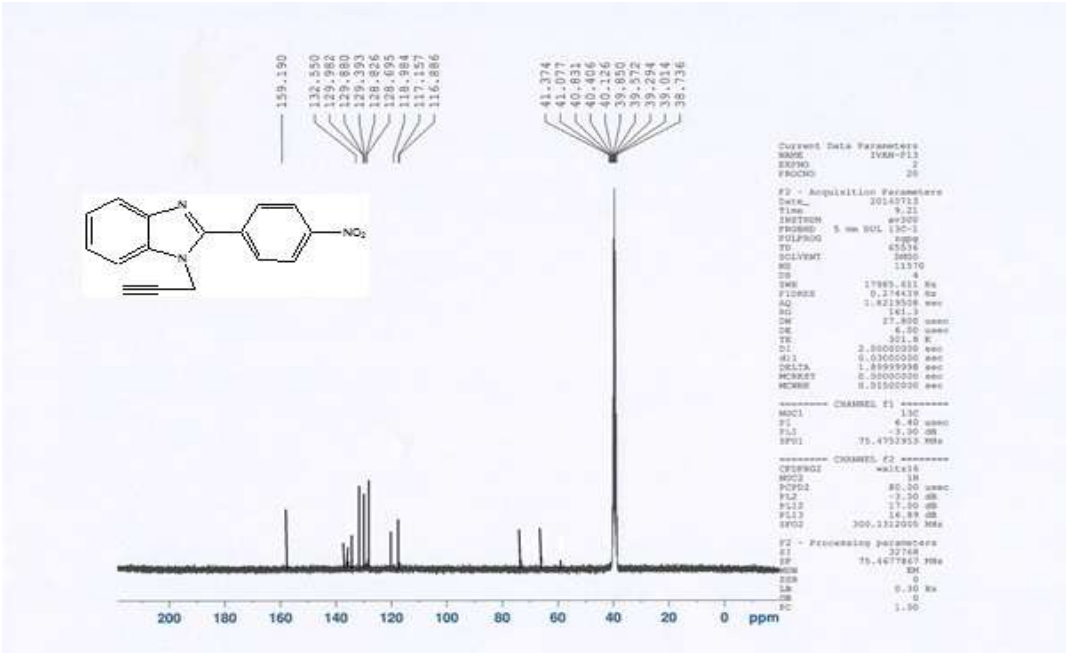
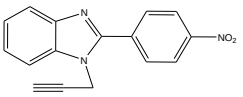
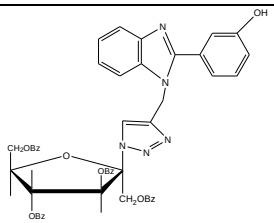
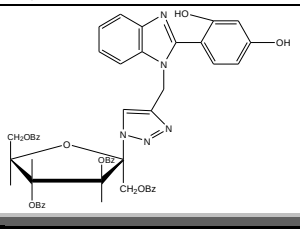
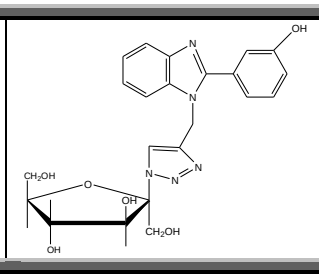


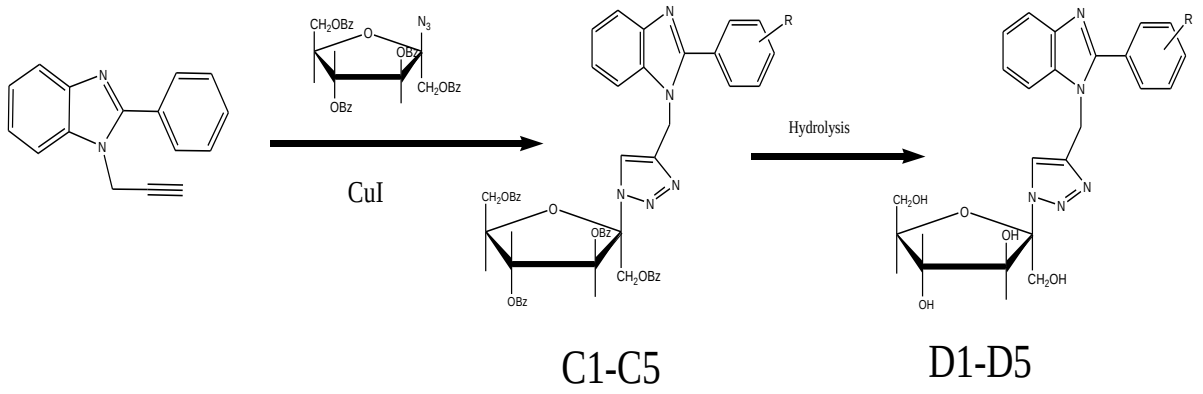
Figure 2- ^{13}C-NMR spectrum for compound B4

Table 5- ^{13} C-NMR spectral data for compounds (B4,C2,C3 and D2)

Comp. No.	**Structures**	**^{13}C-NMR spectral data (δ ppm)**
B4		38.73(CH2, Propargyl); 64.3, 72.45(2C , C≡C); 116.88-132.5 (12C, aromatic) ; 159.19(1C, imidazole)
C2		47.11(CH_2, methylene) ; 63.12-90.75(6C, Sugar carbon); 110.123-131.08 (36C, aromatic);136.02 ,142.92(2C, trizole); 149.69(1C, imidazole);150-153(carbonyl)
C3		41.06(CH_2, methylene) ; 63.5-96.32(6C, Sugar carbon); 128.58-133.67 (36C, aromatic);138.42 ,145.102(2C, trizole); 164.47(1C, imidazole);164.73-165.41(carbonyl)

D2		47.45(CH_2, methylene) ; 61.14-101.91(6C, Sugar carbon); 111-128.71 (36C, aromatic);137.2 ,142.5 (2C, trizole); 149.69(1C, imidazole);157.2(carbonyl)

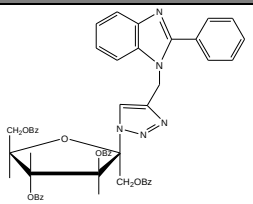
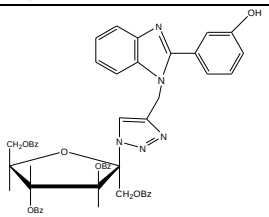
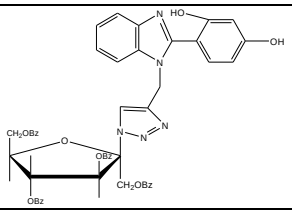
To achieve our synthetic target the nucleoside analogues, the azido sugar (F3) was coupled with modified nucleobase benzimidazole derivative (B1-B5) using Cu^I as catalyst to give the bloked nucleoside (C1-C5) which were debloked with methanolic sodium methoxide to afford our synthetic goal the free nucleoside analogues (D1-D5) Scheme-4.



Scheme 4- Synthesis of protected and free nucleoside analogues.

Some of physical properties and other data of compounds (C1-C5) are listed in Table- 6 and (D1-D5) are listed in Table-7.

Table 6- Physical properties of compounds (C1-C5)

Comp. No.	Compound Structure	Molecular formula	M.wt (g/mol)	m.p	Color
C1		$C_{50}H_{39}N_5O_9$	853	166-170	brown
C2		$C_{50}H_{39}N_5O_{10}$	869	145-149	brown
C3		$C_{50}H_{39}N_5O_{11}$	885	144-148	brown

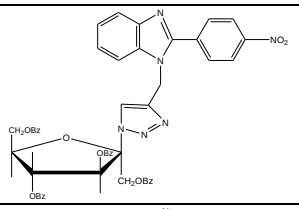
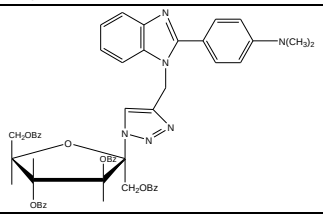
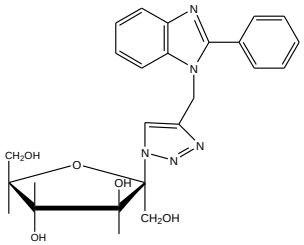
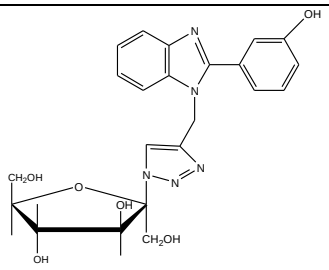
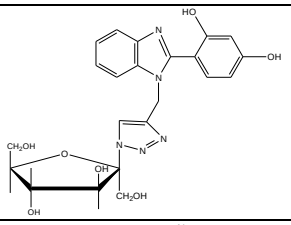
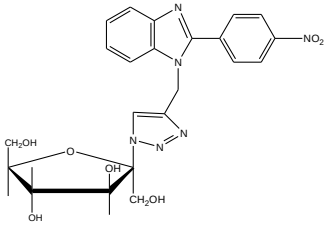
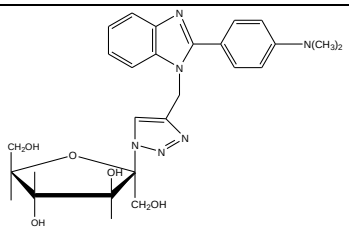
C4		$C_{50}H_{38}N_6O_{11}$	898	170-173	brown
C5		$C_{52}H_{42}N_6O_9$	894	165-168	brown

Table 7- Physical properties of compounds (D1-D5)

Comp. No.	Compound Structure	Molecular formula	M.wt (g/mol)	m.p	Color
D1		$C_{22}H_{23}N_5O_5$	437	65-68	black
D2		$C_{22}H_{23}N_5O_6$	453	66-69	black
D3		$C_{22}H_{23}N_5O_7$	469	45-48	black
D4		$C_{22}H_{22}N_6O_7$	482	70-72	black
D5		$C_{22}H_{28}N_6O_5$	456	76-79	black

The FT_IR spectrum of compounds (C1-C5) (Table-3) showed the characteristic band at 1600 for (N=N) and disappeared of acetylenic and azide bands which indicate that the coupling achieved, ferther evidence is the appearance of carbonyl absorbance at 1720 to 1740 cm^{-1} Figure-3.

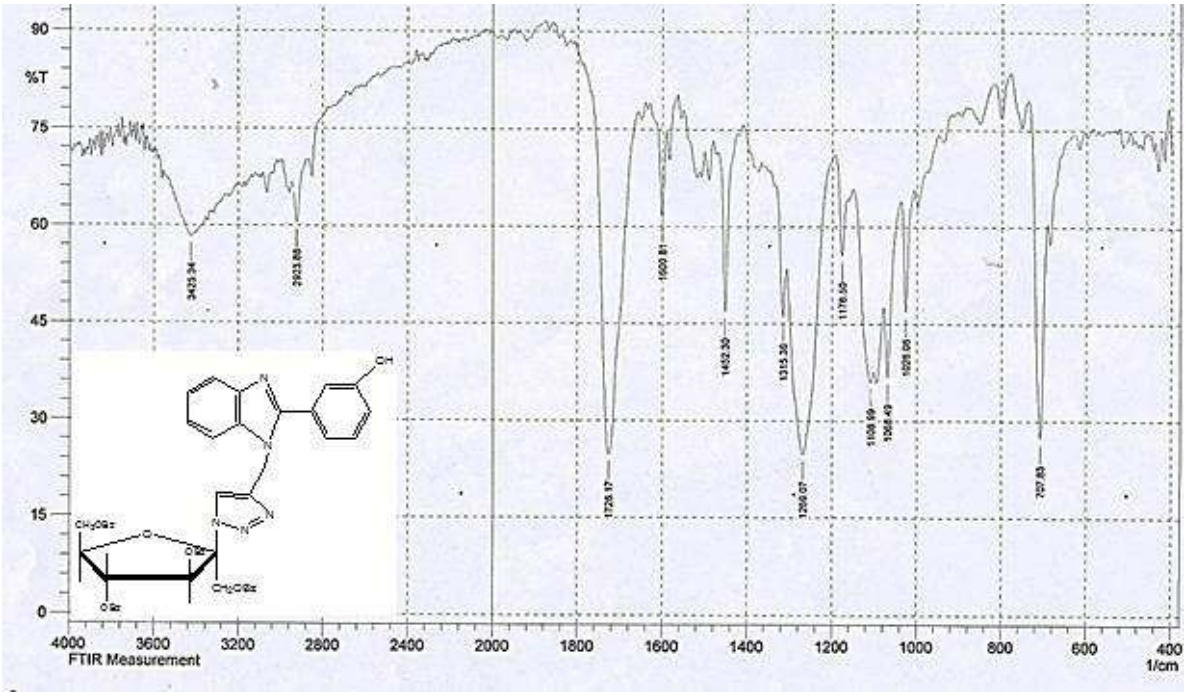


Figure 3- FT-IR Spectrum for compound C2

The FT-IR of free nucleosides (D1-D5) , showed in addition of the above bands, the disappearance of carbonyl bands and appearance of hydroxyl bands between 3420 and 3450 cm^{-1} which is a good evidence for hydrolysis of benzoyl groups.

Compounds C2 and C3 were characterized by ^{1}H-NMR and ^{13}C-NMR , in the ^{1}H-NMR spectra of C2 Table-4 three singlet signals at 2.4 , 3.3 and 9.69 ppm refer to two CH_2 sugar protons , methylene and phenolic protons respectively, while two multiple appear at 4.41-5.66 ppm and 6.37-7.94 ppm for other sugar protons and aromatic protons respectively.

The ^{13}C-NMR spectrum Figure-4 showed the characteristic signals are the imidazole and the carbonyl which appeared at 149.69 ppm and at 150-153 ppm respectively. in addition of that the sugar carbon , aromatic carbons Table-5, ferther more the two signals at downfield 136.02 and 142.92 ppm demonstrate that the sp^1 hybridization converted to sp^2 hybridization .

The ^{1}H-NMR and ^{13}C-NMR spectrum of comp.C3 Table-4 and Table-5 showed the some signals of compound C2 with some shifting either red or blue shift.

Compound D2 was demonstrated also by ^{1}H-NMR and ^{13}C-NMR ,the ^{1}H-NMR spectrum showed four singlet signals at 2.49 , 2.65 , 3.34 and 9.29 ppm for CH_2 sugar protons , methylene protons , hydroxyl groups for sugar moiety and hydroxyl group for phenol respectively . The other sugar prottons appears in the regions between 3.5-5.3 ppm . Aromatic protons appeared as multiplet at 6.15 - 7.96 ppm.^{13}C-NMR spectrum Figure-5 showed the methylene carbon signal at 47.45 ppm, while sugar carbons appeared at the regions between 61.14 -101.9 ppm. The two signals at downfield 137.2 and 142.5 ppm were attributed for the two carbon of trizole ring which indicate there sp2 hybridization. Also the disappeared of carbonyl signal is a good evidence for hydrolysis of blocked nucleoside analogues. The imidazole carbon appeared at 157.2 ppm Table-5.

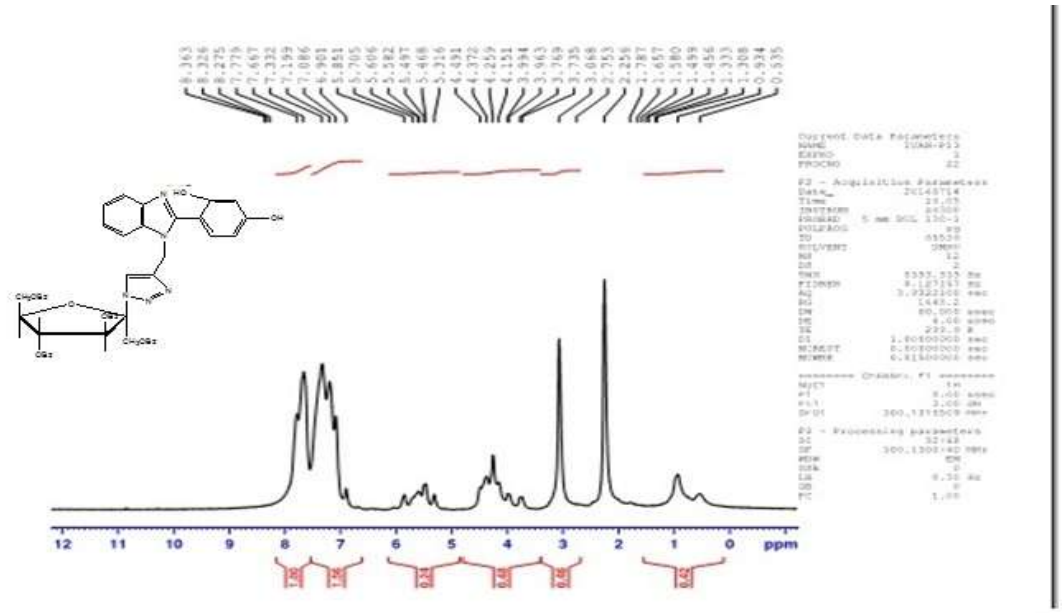


Figure 4- ^{1}H-NMR for C3

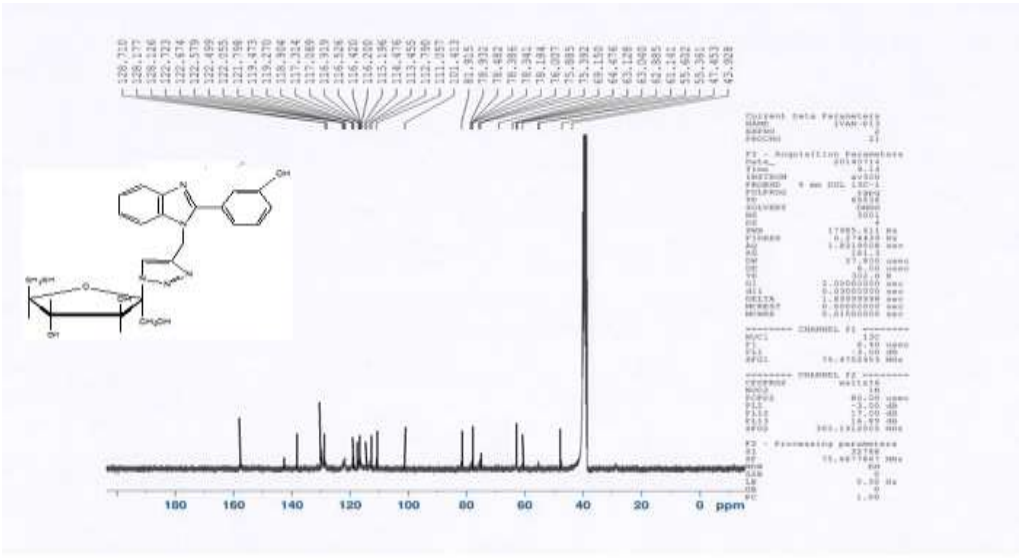


Figure 5- ^{13}C-NMR Spectrum for compound D2

Biological activity

The new free nucleoside analogues (D1-D5) were tested in vitro for antibacterial activity against Gram positive *staph aureas* and *stphylo coccus* , Gram negative *E.coli* and *proteus vulgaris* bacteria and antifungal activity against *candida albicans* . by agar diffution method. The results obtained Table-8 indicate that compounds D3 and D4 showed moderate activity against *staph aureas* and *stphylo coccus* while D1 showed moderate activity against *stphylo coccus* only. D1, D3 and D4 were inactive against Gram negative bacteria while D2 showed moderate activity against *proteus vulgaris*.

All these compounds are compeletely inactive aganst *candida albicans* fungal. The deference of biological activity refers to deferent substituted in the compounds.

Table 8-bioogical activity for compounds (D1-D5)

compounds	**Gram +**		**Gram_**		**fangi**
	Staph	**Strapto**	**E.coli**	**Protus**	**candida**
D1	-	10	-	-	-
D2	-	-	-	10	-
D3	6	10	-	-	-
D4	10	8	-	-	-

References

1. Jordheim, L.P., Fabien Zoulim D.D., and Dumontet, C. **2013**, Advances in the development of nucleoside and nucleotide analogues for cancer and viral diseases. *Nature Reviews Drug Discovery,* 12, pp: 447–464.
2. Huryn, D.M.; Okabe,M. **1992**. AIDS driven Nucleoside Chemistry. *Chem. Rev*.,92, pp1745-68.
3. Townsend, L.B. **1988**.*Chemistry of Nucleosides and Nucleotides*, Plenum Press, New York.
4. Chunxia, C., Chen, C., Bin, L., Jingwei, T., and Jinsong, P. **2012**. Aqueous Synthesis of 1-H-2-substituted benzimidazoles via transition –metal – free intramolcular amination of aryl iodides, *Molecules*. 17, pp:12506-12520.
5. Hasamani, K.M., Shingalapur, R.V. **2011**.Synthesis of 2-Mercaptobenimidzaole Dervatives as Potential Anti-microbial and Cytotoxic Agents. *Arch.Pharm.Chem. Life Sci.*,11, pp: 311-319.
6. Seref, D., Ismail, K., Leyla, Y. **2011**. Microwave supported synthesis of some novel 1, 3-diarylpyrazino[1,2-a]benzimidazole dervatives and investigation of their anticancer activities. *Eur. J. Med. Chem.*, 46, pp: 411-16.
7. Jun, C., Jiangtao, X., Xianjin, L. **2005.** Synthesis and antiviral activity against coxsackkie virus B3 of some novel benzimidazole derivatives. *Bioorg.Med. Chem. Lett.*, 1, pp: 267-269.
8. Labanauskas,L.K., Brukstus, A.B., Gaidelis, P.G., Buchinskaite, V.a., Udrenaite, E.b., Dauksas, V.K. **2000**. Synthesis and Antiinflammatory Activity of some new 1-acyl dervatives of 2-methylthio-5, 6-diethoxy benzimidazole. *Pharm.Chem. J.* 34, pp: 353-355.
9. Gabriel, N. V., Sergio H.F., Mariana T.P., Jorge V.G., Julio Cesar R.L., Samuel E.S., Ismail L.R., Berenice A.G., Yolanda R.G., Rafael V.M., Maximiliano I.B. **2010**.Synthesis , vasorelaxant activity and antihypertensive effect of benzo[d]imidazole derivative, *Bioorg. Med.Chem.* 18, pp: 3985-3991.
10. Warner, W., Dieter, D., Soheila, A., Johannes, G., Bertram, C., and Christos, T. **2004**.Halothiophene benzimidazole as P1 surrogates of inhibitors of blood coagulation factor Xa., *Bioorg. Med. Chem. Lett.* 14, pp: 3763-3769.
11. Kus, C., Kilcilgil, G.A.,Ozbey, S., and Kanak, F.B. **2008**. Synthesis and antioxidant properties of novel N-methyl -1,3,4- thiadiaole -2- amine and 4-methyl -2H-1,2,4-trizole -3(4H)-thione dervatives of benzimidazole class , *bioorg. Med.chem.*8, pp: 4294-4303.
12. Silverstien, R.L., Bassler,G.C., and Morrill, T.C. **1981**. *Spectrometric identification of Organic compounds*. 4th Edition. John Wiley & sons,Inc.,U.S.A.
13. Whistler, R.L. and Wolfrom, M.L. **1962**. *Methods in carbohydrate chemistry,* academic press, New York and London.
14. Swant, R., Kawade, D. **2011**. Synthesis and biological activity of some novel 2- phenyl benzimidazole 1- acetamide dervatives as potential anthelmintic agents. *Acta pharm*. 3, pp: 353-61.
15. Al-Mouamin,Th.M. **2007**. Synthesis of 2, 3-dideoxy- 3,3-gem-di-C-alkyl pyrano-nucleoside analogues, Ph.D. *Thesis* . Department of chemistry, college of science, university of Baghdad, Baghdad, Iraq.
16. Suhadolink, R.J. **1970**. *Nucleoside antibiotics*, John wiley and sons, New York.