Synthesis of 3-C-Spiro Ring Nucleoside Analogues, of Possible Biological Activity

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Abstract

In this study, new derivatives of 3-C-spiro ring nucleoside analogues were synthesized. The structures of these derivatives were characterized by infrared spectroscopy, ^{1}HNMR (some of them) and elemental analysis. The nucleoside derivatives were tested for inhibition of E-coli and were all found to be active.

Introduction

Nucleosides and nucleotides play a key role in many biosynthesis and regulatory process, an extremely important function takes place at the level of structure of ribo – and deoxyribonucleic acids [1] . Several novel 5-substituted 2/ -deoxyuridine analogues were evaluated as substrates for highly purified herpes simplex virus type 1 (HSV-1) encoded thymidine kinase derived from HSV-1 (T K) gene- transfected murine mammary carcinoma cells ,and human platelet thymidine phosphorylase [2,3] .

The potential use of nucleoside analogues in antiparasite chemotherapy is also of great importance and about three billion people in the world are affected by parasitic diseases [4].Protozoan and helmintic parasites have both a deficiency in *denovo* synthesis of purine nucleotides , and the purine salvage pathways are essential for their survival and growth [5,6]. Thus,formycin B [7],allopurinol riboside [8] and thiopurinol riboside [9] figure (1) are converted by a nucleoside phosphotransferase to their corresponding nucleotides , which can either act as inhibitors of other essential enzymes in purine metabolism [6,9] or be incorporated into the nucleic acids of these organisms [8,10] .

Eman [11] has synthesized different derivatives of 3\-alkyl nucleoside analogues [figure 2]. These derivatives were tested for the inhibition of E-coli and were all found to be active.

Spirobicyclic cores display an important role in the field of the development of new bioactive substances [12]. Firas [13] has prepared some derivatives of spiro fused ring and imine position -3 of D-glucofuranose Figure (3) and were all found to have biological activity.

In this work I decided to synthesize new derivatives of nucleoside analogues with spiro ring at position-3 on D- glucofuranose ring.

Experimental

Materials

All chemicals which were used were supplied from Merk chemical Fluka AG, BDH chemicals, Riedel .De Haen AG,Acros Organics, Janssen Chemical and Hopkin and Wiliams. /HNMR spectra were recorded in Hitachi Perkin Emelr ,R-24 B at 60 MHz , elemental analyzer was carried out by using Carlo Erba /Mod 1106 and Infrared spectra were recorded by using Shimadzu -408 .All synthesized compounds were purified by column chromatography by using silica gel (60-120) mesh .The biomaterials were obtained from Biomerieux Ltd.

Synthesis of 1,2:5,6 –Di-*O*-isopropylidene -3-*C*-p-tolouenoyl methyl - α –*D*-allo-hexo-Furanose .(1)

Compund 1 was used as astarting material, this derivative was prepared from the reaction of 3-ulose [14, 15] with p-methylacetophenon according to the reference [11].

Synthesis of 3-p-Tolouenoylelhene -1/2:5, 6-di-*O*-isopropylidne-α-*D*-glucofuranose.(2)

The mixture of dimethyl sulphoxide (40ml), acetic anhydride (20ml), compound 1 (5.0g, 37.26 mmol) is made in a stoppered conical flask, and was stirred at room temperature for (72h) and tlc (benzene: ether 9:1) showed that the reaction was complete .The mixture was diluted with ice water (50ml) and the brown syrup was separated by the decantation and the syrup was washed with ice water (3x20ml), followed by the extraction with chloroform .The organic layer was dried and the solvent was removed under reduced pressure to afford a syrup residue of derivative 2.

Synthesis of 1-[(/1,/2 –*O*-Isopropylidene) ethyl]-3,4-*O*-isopropylidene -8-(/4-tolyl)-2-oxa-6-aza phenyl spiro[4,4] nona -7-imine . (3)

A mixture of 2 (5.0g, 13.36 mmol), phenyl hydrazine (1.4 mL) and (5-10 drops) of diethylamine dissolved in (100mL) absolute ethanol, was refluxed for (48h). The solvent was evaporated and the residue was diluted with water and extracted with chloroform (3x30ml). The organic layer was dried over magnesium sulphate and the solvent was removed to give 3 as asyrup.

Synthesis of 1-[/1,/2 –Dihydroxy ethyl] -3,4,-*O*-isopropylidene -8- (/4-tolyl) -2-oxa-6-aza phenyl spiro [4,4] nona -7-imine .(4)

A solution of 3 (5.0g, 10.77 mmol) in (66%) acetic acid (20mL) was stirred for (24h) at room temperature. The solution was evaporated under reduced pressure and the resulting residue was re-evaporated with toluene twice (20ml) to give 4 as syrup.

Synthesis of 1-[/1-Hyroxy methyl] -3, 4-*O*-isopropylidene -8-(/4-tolyl) – 2-oxa -6-aza phenyl spiro [4, 4] nona-7-imine.(5)

Was added to a well stirred solution of 4 (5.0g, 11.79 mmol) in ethanol (40ml). A saturated solution of sodium hydrogen carbonate (12ml) followed by a solution of sodium periodate (2.5g, 10.75 mmol) in (50ml) water. The mixture was stirred for (1h) at room temperature. Ethylene glycol (2ml) was added, and the solution was further stirred for (5) minutes .Sodium borohydride (0.44g, 11.62 mmol) was added to the resulting aldehydo sugar with continuous stirring for (1h). The reaction mixture was filtrated and extracted with chloroform .The organic layer was dried and evaporated to give 5 as syrup.

Synthesis of 1-[/1 – Benzoyl methyl] -3, 4-*O*- isopropylidine -8-(/4-tolyl) -2-oxa -6-aza phenyl spiro [4, 4] nona -7-imine. (6)

Compound 5 (4.0g,10.15 mmol) in anhydrous benzene (60 ml)containing pyridine (6mL) was benzoylated with benzoyl chloride (1.42 ml,10.10 mmol).After stirring for (24h) at room temperature ,the mixture was poured into ice water (100ml) .The organic layer was separated and washed with water (3x30ml) ,dried and evaporated to give 6 as syrup.

Synthesis of 1-[/1-Benzoyl methyl]-8- (/4-tolyl)-2-oxa-6-aza phenyl spiro [4, 4] nona -7-imino -3, 4-di-*O*-triflouro acetate. (7)

water (2ml) and trifluoroacetic acid (20ml) was added to a solution of 6(5.0g,10.04mmol) in acetic acid (20ml) ,.The resulting mixture was stirred for (5h) at room temperature .The reaction mixture was then neutralized with solid sodium hydrogen carbonate and extracted with dichloromethane (2x100ml).The combined extracts were dried and the solvent was removed to give a syrup .This syrup was immediately treated with trifluoroacetic anhydride (4ml) in a mixture of anhydrous benzene (40ml) and anhydrous pyridine (8ml) with stirring for (24h) at room temperature .Ice water was added to the mixture and the organic layer was separated ,dried and concentrated under reduced pressure .Traces of pyridine were removed by co -evaporation with dry toluene (2x30 ml) to give 7 as syrup .

Synthesis of 7 [/1-Benzoyl methyl] -/4-*O*-trifloouro acetyl -/8-(4-tolyl) -/2-oxa-/6-aza phenyl spiro [4, 4] nona-/7- imino] theophylline .(8)

The theophylline mercury salt 9 was synthesized according to the reference [16]. This salt(0.56 g,1.54 mmol) was powdered ,suspended in (50ml) sodium dried xylene and the solvent was distilled of to remove the traces of water azeotropically .When the temperature of mixture was raised to 137c^{o} ,the suspension was allowed to cool (below 50c^{o}) .Compound 7 (1g,1.53 mmol) in xylent (20mL) was added to the suspension and refluxed with stirring for (30 h) .The traces of theophylline salt was filtrated from the hot xylene and washed with dichloromethane (20ml) .The organic layer was dried and removed to give acetylated nucleoside 8 as a syrup .^{1}HNMR ($CDCL_3$) δ:2.5(3H,S,CH_3Ar) ; 2.82(2H,s,CH_2-$^{/}$9); 3.2,3.4(6H,s,s,2NCH_3); 3.8-4.9 (4H,m,H-$^{/}$4,H-$^{/}$1 and H^{a},H^{b} CH_2OB_Z); 5.9 (1H,d,H-$^{/}$3), 6.8-8.8 (15H,m,ArH and base) .

Synthesis of 7[($^{/}$1-Hydroxy methyl) -$^{/}$4-hydroxy -$^{/}$8-(4-tolyl)-$^{/}$2-oxa-$^{/}$6-aza phenyl spiro [4,4] nona-$^{/}$7-imino] theophyllene.(10)

Compound 8(0.5 g, 0.69 mmol) and sodium methoxid (0.3g, 5.55 mmol) were dissolved in ethanol (30mL) .The solution was stirred under reflux for (24h) .The solvent was removed to give 10 as a syrup.

Synthesis of 1[($^{/}$1-Benzoyl methyl)-$^{/}$4-*O*-triflouro acetyl-$^{/}$8-(4-tolyl) -$^{/}$2-oxa-$^{/}$6- aza phenyl spiro [4, 4] nona -$^{/}$7-imino] uracil. (12)

Anhydrous stannic chloride (0.6 ml) and few pellets of molecular sive 4A was added to a mixture of 7 (2.0g,3.07 mmol) and silylated uracil 13 (0.8g,3.12 mmol)[compound 13 was synthesized by using the reference [17]] in (40ml) of anhydrous dichloromethane.The mixture was stirred at 20c^{o} for (24h)The reaction mixture was poured in to an excess of sodium bicarbonate solution and extracted with dichloromethane (3x40ml) .The organic layer was dried and removed to give 12 as semi solid .^{1}HNMR ($CDCL_3$).δ:2.4(3H,s,CH_3Ar) ;2.7 (2H,s,CH_2-$^{/}$9) ;3.7-4.9 [6H,m,H-$^{/}$1,H-$^{/}$4,H-4,H-5,and (H^{a},H^{b}) CH_2-OB_Z];5.8(1H,d,H-$^{/}$3) ; 6.5(1H,b,NH) ;6.9-8.9(14H,m,ArH).

Synthesis of 1[($^{/}$1-Hydroxy methyl) -$^{/}$4-hydroxy -$^{/}$8- (4-tolyl) -$^{/}$2-oxa-$^{/}$6-aza phenyl spiro [4, 4] nona -$^{/}$7-imino] uracil. (14)

Flowing the same procedure of the preparation of compound 10, the compound 12 (0.5g, 0.74 mmol) and sodium methoxide (0.3g, 5.55mmol) were dissolved in ethanol (30mL). The reaction mixture was stirred under reflux for (18h) to give 14 as a syrup.

Results and Discussions

Compound (1) was chosen as a starting material for the synthesis of a new derivative of 3-C-spiro ring nucleoside analogues .The strategy used for the synthesis of 2, 3, 4, 5,6,7,8,10,11,12 and 14 was started with derivative 1 in a series of reactions [scheme 1]. Compound 2 was synthesized by the dehydration of 1 with DMSO / Ac_2O [18, 19] and gave 2. The IR spectra of 2 showed the disappearance of stretching band at 3360cm^{-1} for hydroxyl group with appearance of stretching band at 1640cm^{-1} for (C=C).Tables (1) and (2) showed the characteristic IR absorption bands and physical properties for all of the new derivations .The α , β-unsaturated branched chain 2 undergoes 1/3-and adding the diamine (phenyl hydrazine) to give the spiro derivative 3 [20,21].Compound 3 was characterized by IR and elemental analysis .The IR spectra of 3 showed the disappearance of stretching band at 1640 cm^{-1} for (C=C) and disappearance of (CO) group at 1690cm^{-1}.To obtain the derivative 5 ,the isopropylidene acetal at C-$^{/}$1 and C-$^{/}$2 was removed with acetic acid followed by periodate oxidation and borohydride reduction .The $^{/}$1-hydroxyl group was protected with benzoyl group by using benzoyl chloride to give the derivative 6.The IR spectra of 6 showed a stretching band at 1700cm^{-1} for (CO) group with the disappearance of (OH) group at 3385cm^{-1}.Compound 6 was treated with a mixture of trifluoroacetic acid and acetic acid followed by the reaction with trifluoroacetic anhydride gave 7. Compound 8 was prepared by using the Koenigs-Knorr condensation method [22,23].The ^{1}HNMR spectrum of 8 showed a singlet at 2.5 for p-methyl group ,singlet at 2.82 for methylene group (CH_2-9$^{/}$),two singlet at 3.2 ,3.4 for (2NCH_3) , multiplet at 3.8-4.9 for (H-4$^{/}$,H-1$^{/}$and H^{a} , H^{b} CH_2OB_z) ,doublet at 5.9 for H-3$^{/}$ and

multiplet signals at 6.8-8.8 for aromatic rings protons and base. The treatment of compound 8 with sodium methoxide in ethanol under reflux [24]gave 10.Compound 7 was treated with silylated uracil [17] gave12.The suggested mechanism of the nucleoside formation 12 is given below [scheme 2].The ^{1}HNMR spectrum of 12 showed a singlet at 2.4 for p-methyl group , singlet at 2.7 for methylene group , multiplet at 3.7-4.9 for H-/1 , H-/4 , H-4 , H-5 and H^a, H^b for CH_2-OB_z , doublet at 5.8 for H-/3 ,broad signal at 6.5 for NH uracil and multiplet at 6.9-8.9 for aromatic protons. The treatment of compound 12 with sodium methoxide in ethanol gave 14 as a syrup .Compound 10 and 14 exhibited a biological activity against E-coli bacteria. Compound 10 exhibited ahigher degree of activity than the other (table 3).

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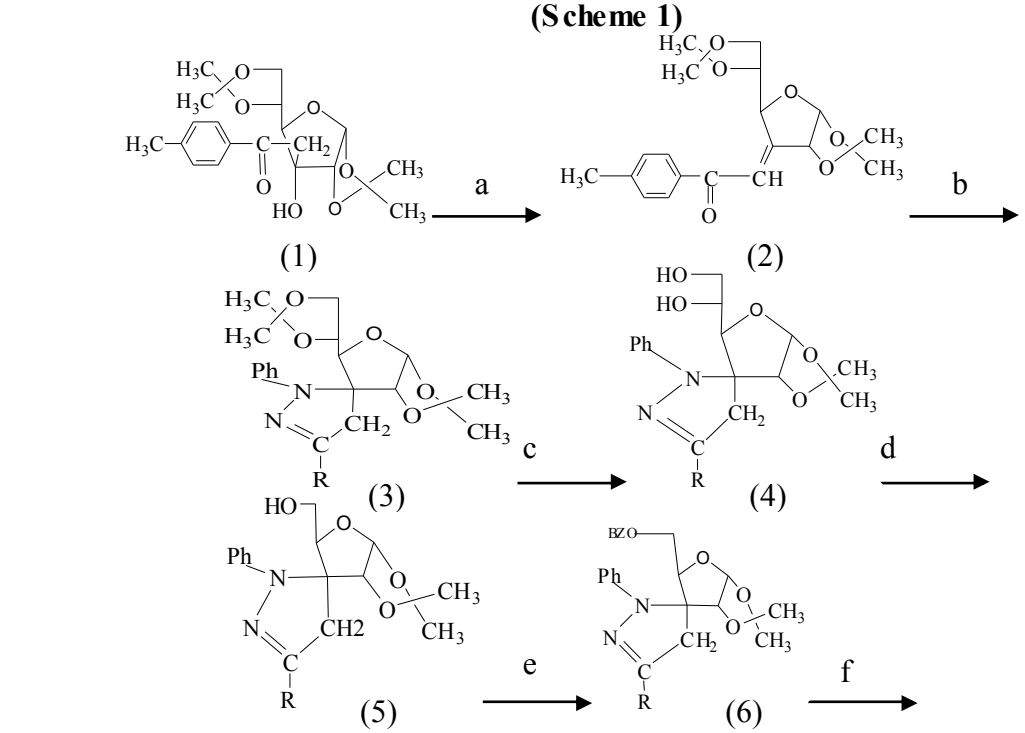
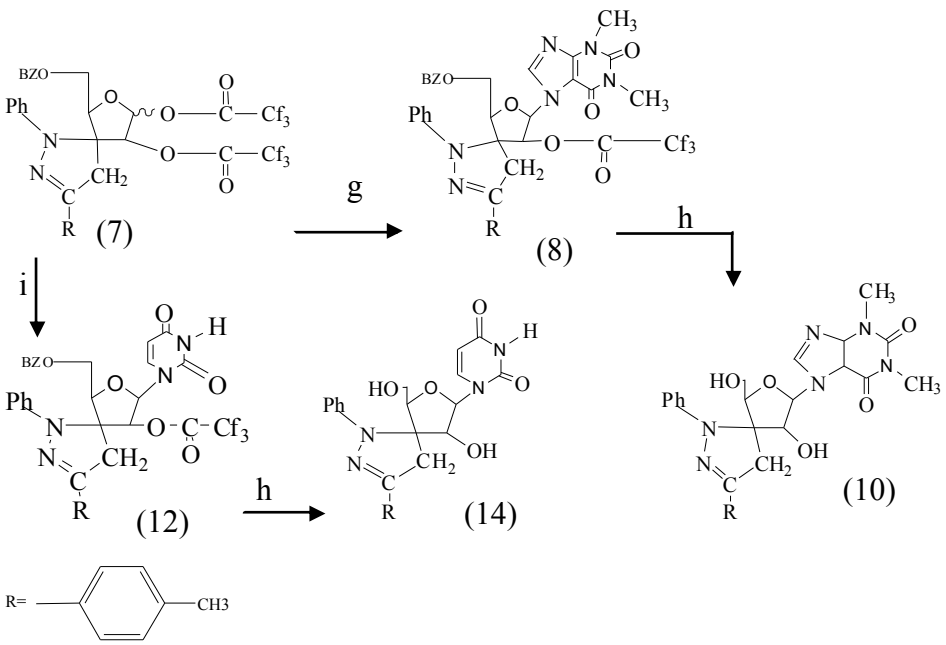
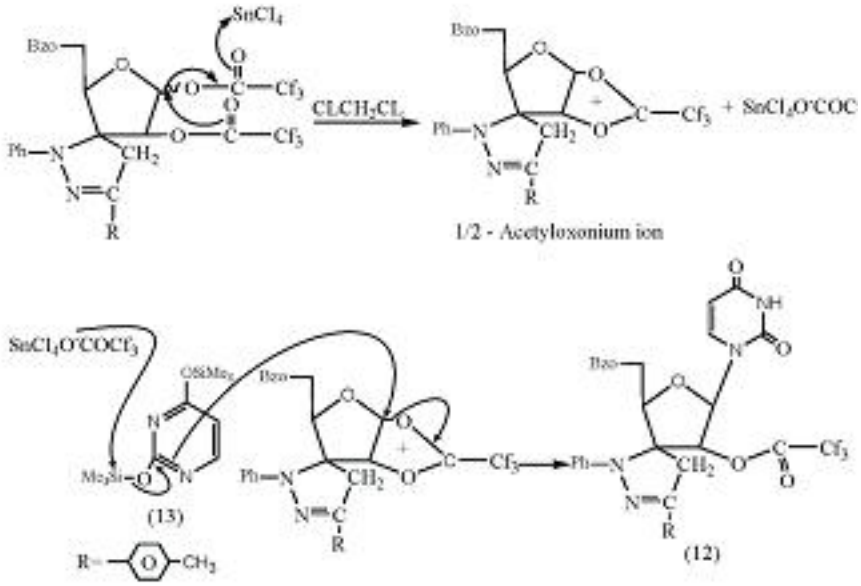
(Scheme 1)

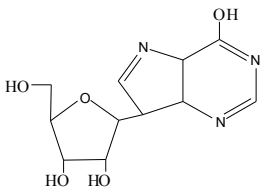




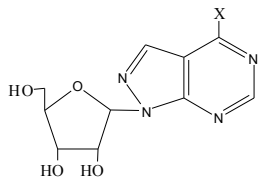
a: DMSO, Ac_2O.
b: Phenyl hydrazine, Diethyl amine, Absolute ethanol.
c:66%AcOH, d: $NaIO_4$ then $NaBH_4$.
e: BzCl,Pyridine, Benzene.
f:CF_3COOH, CH_3COOH, H_2O than $(CF_3CO)_2O$,Benzene,Pyridine.
g: Theophylline Mercuric salt, Xylene, Δ .
h: CH_3ONa, C_2H_5OH, reflux.
i: Silylated Uracil, CH_2Cl_2.

(Scheme 2)



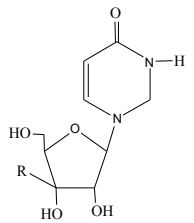


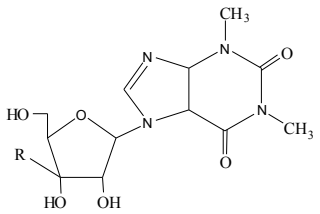
Formycin B



Allopurinol riboside (X=OH)
Thiopurinol riboside (X=SH)

Fig.1





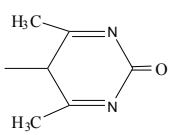
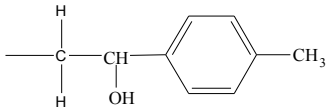
R = ( , )

Fig.(2)

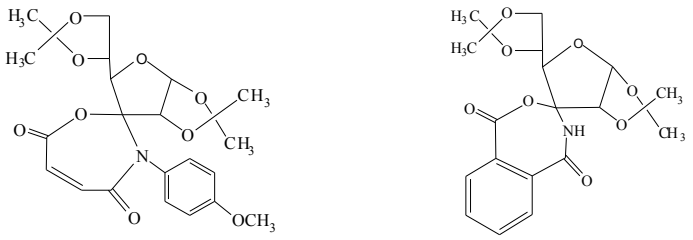


Fig.(3)

Table (1): Characteristic IR absorption bands of the new derivatives

Compound No.	Infrared data (υ_{max} cm^{-1}) (film)
2	(C=C) stretching aliphatic 1640;(CO)1690; (C=C) aromatic ring 1480 – 1610; (C=C) bending 815
3	(C=C) aromatic ring 1460-1630;(C=N) 1660 ;(C=C) bending 780.
4	(OH) 3400 ; (C=C) aromatic ring 1480-1640,(C=N) 1660; (C=C) bending 800.
5	(OH) broad 3385;(C=C) aromatic ring1500-1620;(C=N) 1640 (C=C) bending 815.
6	(CO)1700;(C=C) aromatic ring 1480-1610; (C=N)1630;(C=C)bending for p-substitutions ring 830 ;(C=C)bending for benzoyl ring 670.
7	(CO) for OBz 1710;(CO) for CO_2Cf_3 1690;(C=C) aromatic ring 1460-1610;(C=N) 1630;(C=C) bending 810-720.
8	(CO) for OBz and CO_2Cf_3 1700,1685;(CO) for CONH 1675;(C=C) aromatic ring 1445-1620;(C=N) 1640 (C=C) bending 700,800.
10	(OH)3340,(CO) 1680;(C=C)1520-1600;(C=N)1610; (C=C) bending 820.
12	(NH)3410;(CO) for OBz andCO_2Cf_3 1700,1680;CONH 1670;(C=C),aromatic ring 1460-1610;(C=N)1620 (C=C) bending 710,825.
14	(NH,OH)3200,3400;(CO)1670; (C=C) aromatic ring 1500-1610;(C=N)1620 ;(C=C) bending 810.

Table (2): Physical properties and elemental analysis for new derivatives.

Formula & Comp. No.	**Physical state**	**Rat of flow in thin lyer chromatography (tlc),R_f (solvent)**	**Elemental analysis calculated(found)**			
			C%	H%	N%	Yield %
$C_{21}H_{26}O_6$ (2)	Syrup	0.62 (benzene: ether 9:1)	67.37 (67.1)	6.95 (7.15)		65
$C_{27}H_{32}N_2O_5$ (3)	Syrup	0.57 (benzene:n-hexane9:1)	69.82 (70)	6.89 (6.77)	6.03 (5.89)	50.27
Formula & Comp. No.	**Physical state**	**Rat of flow in thin lyer chromatography (tlc),R_f (solvent)**	**Elemental analysis calculated(found)**			
			C%	H%	N%	Yield %
$C_{24}H_{28}N_2O_5$ (4)	Syrup	03.5 (benzene; methanol 9.5 :0.5)	67.92 (68.12)	6.6 (6.15)	6.6 (6.35)	60.55
$C_{23}H_{26}N_2O_4$ (5)	Syrup	0.42 (benzene; methanol 9:0.5)	70.05 (70.3)	6.59 (6.25)	7.10 (6.88)	30.2
$C_{30}H_{30}N_2O_5$ (6)	Syrup	0.72 (benzene;ethyla cetate 9.5;0.5)	72.28 (71.91)	6.02 (6.04)	5.62 5.33) (	67.8
$C_{31}H_{24}N_2O_7F_6$ (7)	syrup	0.65 (benzene: methanol 9:0.5)	57.23 (56.89)	3.69 (3.49)	4.3 3.98) (	48
$C_{36}H_{31}N_6O_7F_3$ (8)	syrup	0.48 (benzene: methanol 9:0.5)	57.23 (56.89)	3.69 (3.44)	4.3 (3.98)	71.5
$C_{27}H_{28}N_6O_5$ (10)	syrup	0.33 (benzene: methanol 9:0.5)	62.79 (63.11)	4.32 (4.59)	11.73 (11.53)	55
$C_{33}H_{27}N_4O_7F_3$ (12)	Semi solid	0.52 (benzene: methanol 9:0.5)	61.11 (61.46)	4.16 (3.86)	8.64 (8.28)	79.2
$C_{24}H_{24}N_4O_5$ (14)	syrup	0.4 (benzene: methanol 9:0.5)	64.28 (64.81)	5.35 (4.97)	12.5 (12.21)	42.3

Table (3):Effect of antimicrobial agents on Escherichia Coil

Com. No.	Effect of nucleoside derivatives on the growth of E-coli bacteria											
	1	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1	0.09	Con. gm/ml
10	_	_	_	_	_	_	_	_	_	_	+	
14	_	_	_	_	_	_	_	+				
U. base	+											
T. base	+											
Blank	+											

(-) No growth, U=Uracil

(+) Growth , T=Theophylline

تحضير ممثلات نيوكليوسيد من نوع C-3–حلقة سبايرو ذو فعالية بايولوجية محتملة

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الخلاصة

تم في هذا البحث تحضير مشتقات جديدة لمماثلات النيوكليوسيد تحتوي على حلقة سبا يرو في موقع 3اذ اختير المشتق 1مادة ابتدائية لغرض تحضير مماثلات النيوكليوسيد بعد مروره بسلسله من التفاعلات ، اذ تم مفاعله المشتق 1مع ثنائي مثيل السلفوكسيد وانهيدريد الخليك لغرض سحب جريئة ماء من الموقع ثلاثة للحصول على المشتق 2. لغرض الحصول على حلقة سبايرو في موقع ثلاثة تمت مفاعلة المشتق 2 مع الفنيل هيدرازين ،اذ أعطى المشتق 3. ازيحت مجموعة الحماية (الايزوبروبيلدين) في الموقع 1و2 باستعمال حامض الخليك تبعتها عملية أكسدة واختزال للحصول على المشتق 5. أن حماية مجموعة الهيدروكسيل في الموقع –1 باستخدام كاوريد البنزويل يوفر الفرصة المناسبة لإزاحة مجموعة الاسيتال في الموقع –3 و– 4 ،اذ أعطى المشتق 6. عومل المشتق 6 بمزيج من حامض الخليك وثلاثي فلوريد الخليك تبعه التفاعل مع ثلاثي فلوريد انهيدريد الخليك لإعطاء المشتق 7. تمت مفاعلة المشتق 7 مع ملح الزئبق للثيوفلين الذي أعطى مماثل النيوكايوسيد 8. أن تفاعل المشتق 7 مع مشتق السيلايل لليوراسيل أعطى مماثل النيوكليوسيد 12. أن تسخين المشتقين 8و12 كل على انفراد مع ميثوكسيد الصوديوم في الميثانول أعطى مشتقات النيوكليوسيد الحرة 10 و 14 على التوالي . تمت دراسة الفعالية البايولوجية للمشتقات 10 و 14 ضد بكتريا القولون وقد وجد أن لها فعالية بايولوجية وأكثرها فاعلية كان المشتق 10.