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SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF NEW NUCLEOSIDE ANALOGUES FROM BENZOTRIAZOLE

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Abstract: Novel derivatives of 1-(′1, ′3, ′4, ′6-tetra benzoyl-*β*-D-fructofuranosyl)-1H- benzotriazole and 1-(′1, ′3, ′4, ′6-tetra benzoyl-*β*-D-fructofuranosyl)-1H-benzotriazole carrying Schiff bases moiety were synthesised and fully characterised. The protection of D-fructose using benzoyl chloride was synthesized, followed by nucleophilic addition/elimination between benzotriazole and chloroacetyl chloride to give 1-(1- chloroacetyl)-1H-benzotriazole. The next step was condensation reaction of protected fructose and 1-(1-chloroacetyl)-1H-benzotriazole producing a new nucleoside analogue. The novel nucleoside analogues underwent a second condensation reaction with different aromatic and aliphatic amines to provide new Schiff bases. The prepared analogues were characterised by FT-IR, ^{1}H NMR, ^{13}C NMR, HRMS(EI^{+}) spectra. These analogues were tested against different bacteria to evaluate them as antimicrobial agents.

Keywords: antimicrobial agents, benzotriazole, nucleoside analogues, protected fructose, Schiff bases.

1. Introduction

Nucleoside belongs to a class of organic compounds with their structures being composed of a nitrogen-containing heterocyclic nucleobase and 5-carbon sugar. The nucleobase is bound to the 5-carbon sugar, either a ribose or a deoxyribose, through a *β*-glycosidic linkage [1, 2].

Nucleoside analogues play a significant role in pharmacologic activity [3] and some of them show high effectiveness as antiviral and antitumoral agents [4]. Other nucleoside analogues play important role as therapeutic agents [5]. Variety of functionalities has been introduced into their ribose moiety or the heterocyclic moiety [6]. Modified nucleosides and nucleobases are pharmacologically diverse family, which include anticancer and antiviral compounds [7, 8].

Benzotriazole features two fused rings, which can represent mimic purine base in nucleoside of DNA and RNA strains; benzotriazole derivatives have chemical and biological properties that are versatile in the pharmaceutical industry. These derivatives act as agonists for many proteins and also are used in photographic developers and emulsions as restrainers [9, 10].

So Schiff base can be synthesised from an aliphatic or aromatic amine and a carbonyl compound by nucleophilic addition forming imine compounds [11]. Schiff bases are common enzymatic intermediates where an amine, such as the terminal group of lysine residue reversibly reacts with aldehyde or ketone of cofactor or substrate. The common enzyme cofactor PLP forms a Schiff base with lysine residue and is transaldiminated to the substrate. Similarly, the cofactor retinal forms a Schiff base in rhodopsins, including human rhodopsin (*via* Lysine 296), which is key in the photoreception mechanism [12]. A new methodology to synthesize new modified nucleoside analogues using protected fructose and benzotriazole derivative was developed in this article, introducing Schiff base moiety. The new analogues were examined against different types of bacteria to evaluate them as antimicrobial agents.

2. Experimental

All used chemicals in this article were purchased from Sigma-Aldrich unless otherwise stated. Triethyl amine, DCM, and $CHCl_3$ were distilled from calcium hydride under nitrogen. ^{1}H-NMR spectra were measured on Bruker Avance 500 MHz spectrometer or Bruker Avance DPX 400 NMR spectrometer. ^{13}C-NMR spectra

were measured on a Bruker Avance 500 MHz spectrometer and are reported as chemical shift downfield from tetramethylsilane, coupling constant where appropriate and assignment. It is worth mentioning that ^{1}H-NMR and ^{13}C-NMR spectra and GC-MS (EI^{+}) were measured at Cardiff University in the United Kingdom. IR spectra were recorded on Perkin ELMER 1600 series FT-IR spectrometer, and samples were prepared as thin films of neat liquid on NaCl discs for oils and as KBr disks for solids. EI^{+} mass spectra were measured on a Micromass LCT premiere XE mass spectrometer, ES^{-} mass spectra were provided by the UK EPSRC mass spectrometry service. The purity of purified compounds was judged to be > 95 % by TLC and/or GC analyses and NMR spectroscopic analyses. TLC analyses were performed on plates pre-coated with 250 µM layers of either silica gel 60 F254 or alumina 60 F254. All retention factors (R_f) are on silica gel and alumina TLC plates. TLC visualisations were performed with 5 % phosphomolybdic acid, CAM (ceric-ammonium-molybdate), $KMnO_4$, I_2 vapor, or UV light.

2.1. Protection and Preparation of Sugar Moiety: 1,3,4,6-tetra-O-benzoyl-β-D-fructofuranose 2 [13]

Benzoyl chloride (7 ml) was added dropwise to anhydrous-D-fructose (2 g, 11.11 mmol) that suspended in mixture of chloroform (30 ml) and dry pyridine (5 ml), after that the mixture was heated at 318–338 K with continuous stirring for 4 h. The resulted reaction was indicated by TLC. The mixture was poured over ice-water and then extracted with $CHCl_3$ (3×15 ml). The organic layer was washed with 5 % HCl solution (10 ml) to remove the excess of pyridine. The organic layer was naturalized with 5 % of sodium carbonate solution (10 ml), after that the organic layer was dried over sodium sulphate and the solvent was evaporated under vacuum to give a syrup that crystallized from absolute ethanol to afford white crystals (m.p. 394–395 K); IR (thin film) cm^{-1} 3217 (broad s) (OH, stretch), 3064 (m) (CH aromatic, stretch), 2918 (m) (CH aliphatic, stretch), 1720 (s) (C=O ester, stretch), 1655 (m) (C=C aromatic, stretch); ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 2.1-2.3 (1H, s, 1OH), 3.0-3.1 (4H, s, $2CH_2OBz$), 3.3-3.4 (2H, s, 2CH), 7.3-7.4 (8H, m, 8CH aromatic), 7.5-7.6 (4H, m, 4CH aromatic), 7.8-7.9 (8H, m, 8CH aromatic); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C 64 ($2CH_2O$), 70 (2CH), 92 (2COH), 130 (8CH aromatic), 134 (8CH aromatic), 136 (4CH aromatic), 138 (4CC=O), 170 (4C=O); HRMS(EI^{+}) found 612.2303, $C_{34}H_{28}O_{11}$ requires 612.2301.

2.2. Preparation of 1-(1- chloroacetyl)-1H-benzotriazole 4 [14]

Chloroacetyl chloride (0.01 mol, 0.79 ml) was added dropwise to a mixture of benzotriazole (0.01 mol, 1.19 g), triethyl amine Et_3N (1 ml) in DMF (20 ml). The reaction mixture was stirred for 3 h, after that the mixture was filtered, dried and recrystallized from ethanol to give dark yellow oil (85 %), R_f = 0.75 (benzene:methanol = 4:7); IR (thin film) cm^{-1} 3064 (m) (CH aromatic, stretch), 2980 (m) (CH aliphatic, stretch), 11672 (s) (C=O amide, stretch), 1655 (m) (C=C aromatic, stretch), 1515 (m) (N=N, stretch), 1250 (m) (C–N, stretch); ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 3.4-3.5 (2H, s, $ClCH_2CO$), 7.5-7.6 (2H, t, 2CH aromatic), 7.8-7.9 (2H, d, 2CH aromatic); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C 45 (CH_2CO), 119 (2CH aromatic), 128 (2CH aromatic), 147 (2CN aromatic), 190 (C=O); HRMS(EI^{+}) found 195.341, $C_8H_6ClON_3$ requires 195.342.

2.3. General Procedure for the Preparation of Protected Nucleoside Analogues of 1-(′1, ′3, ′4, ′6-tetra benzoyl-β-D-fructofuranosyl)-1-H- benzotriazole 5 [15]

1,3,4,6-Tetra-O-benzoyl-β-D-fructofuranose **2** (3.5 g, 0.1 mol), silver oxide (25 g, 0.11 mol), calcium sulphate (10 g, preheated for 2 h at 513 K) and dry, absolute ethanol (10 ml) are placed in a 500 ml, two necked, round-bottomed flask equipped with a drying tube and a dropping funnel. The flask is wrapped in black paper to protect the contents from light. The flask contents were stirred about 1 h to ensure total absence of water. Iodine (5 g) was later added as a catalyst. 1-(1-Choro acetyl-1-H-benzotriazole **4** (4.3 g, 0.1 mol), was dissolved in absolute ethanol (10 ml) and added through dropping funnel to the stirred reaction mixture over a period of about 1 h. The stirring continued for 24 h. The reaction mixture then was filtered through a layer of a filter pad, and the residue was washed well with chloroform. The filtrate and washings were combined and concentrated under reduced pressure. The residue was re-crystallised from chloroform to give brown dark powder in the yield 75 % (m.p. 470–472 K), R_f = 0.75 (benzene: methanol = 6:4); IR (thin film) cm^{-1} 3070 (m) (CH aromatic, stretch), 2925 (m) (CH aliphatic, stretch), 1720 (s) (C=O ester , stretch), 1672 (s) (C=O amide, stretch), 1650 (m) (C=C aromatic, stretch), 1515 (m) (N=N, stretch; ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 3.0 (4H, s, $2CH_2OBz$), 3.3 (2H, s, CH_2CO), 3.5 (2H, s, 2CH), 7.2-7.3 (8H, m, 8CH aromatic), 7.5-7.6 (4H, m, 4CH aromatic), 7.5-7.6 (2H, t, 2CH aromatic), 7.8-7.9 (8H, m, 8CH

aromatic), 7.95-8.0 (2H, d, 2CH aromatic); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C 45 (CH_2CO), 64 ($2CH_2O$), 70 (2CH), 92 (2COH), 119 (2CH aromatic), 128 (2CH aromatic), 130 (8CH aromatic), 134 (8CH aromatic), 136 (4CH aromatic), 138 (4CC=O), 147 (2CN aromatic), 170 (4C=O), 190 (C=O) ; HR-MS(EI^+) found 806.125 $C_{42}H_{33}O_{12}N$ requires 806.123.

2.4. Preparation of Schiff base of 1-[(methylene-benzimine)-O-(3`, 4`, 6`-tribenzoyl- β-D-fructofuranosyl]-1H-benzotriazole 6 [16]

The amounts of nucleoside analogue **5** (2 mmol, 0.4 g) and aniline (2 mmol, 2 ml) were dissolved in dichloromethane DCM (10 ml) and glacial acetic acid (2 ml). The mixture was heated under reflux for 1.5 h. The mixture was neutralized with $NaHCO_3$ (10 ml) and extracted with $CHCl_3$. The organic layer was washed with water (10 ml) and dried over sodium sulphate. The solvent was evaporated to give the dark brown crystal (m.p. 453 K, decomposition); R_f = 0.75 (benzene: methanol = 6:4); IR (thin film) cm^{-1} 3070 (m) (CH aromatic, stretch), 2925 (m) (CH aliphatic, stretch), 1720 (s) (C=O ester , stretch), 1650 (m) (C=C aromatic, stretch), 1600 (m) C=N imine, stretch), 1512 (m) (N=N, stretch); ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 3.0-3.2 (4H, s, $2CH_2OBz$), 3.3 (2H, s, CH_2CN), 3.5 (2H, s, 2CH), 7.2-7.3 (8H, m, 8CH aromatic), 7.5-7.6 (4H, m, 4CH aromatic), 7.5-7.6 (2H, t, 2CH aromatic), 7.7-7.75 (5H, m, 5CH aromatic), 7.8-7.9 (8H, m, 8CH aromatic), 7.95-8.0 (2H, d, 2CH aromatic); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C 45 (CH_2CO), 64 ($2CH_2O$), 70 (2CH), 92 (2COH), 119 (2CH aromatic), 128 (2CH aromatic), 130 (8CH aromatic), 132 (5CH aromatic), 134 (8CH aromatic), 136 (4CH aromatic), 137 (C aromatic), 138 (4CC=O), 147 (2CN aromatic), 170 (4C=O), 190 (C=O) ; HRMS(EI^+) found 795. 223 $C_{48}H_{38}O_{11}N$ requires 795.223.

2.5. Preparation of Schiff base of 1-[{(methylene)-3-bromo-benzimine}-O-(3`, 4`, 6`-tribenzoyl- β-D-fructofuranosyl]-1H- benzotriazole 7

The amounts of nucleoside analogue **5** (2 mmol, 0.4 g) and *0*-bromo aniline (2 mmol, 2 ml) were equally dissolved in DCM (10 ml) and glacial acetic acid (2 ml). The mixture was heated under reflux for 1.5 h. The mixture was neutralized with $NaHCO_3$ (10 ml) and extracted with $CHCl_3$. The organic layer was washed with water (10 ml) and dried over sodium sulphate. The solvent was evaporated to give the dark red oil (m.p. 453 K, decomposition); R_f = 0.75 (benzene: methanol = 6:4); IR (thin film) cm^{-1} 3070 (m) (CH aromatic, stretch), 2925 (m) (CH aliphatic, stretch), 1720 (s) (C=O ester , stretch), 1650 (m) (C=C aromatic, stretch), 1600 (m) C=N imine, stretch), 1512 (m) (N=N, stretch) ; ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 3.0-3.2 (4H, s, $2CH_2OBz$), 3.3 (2H, s, CH_2CN), 3.5 (2H, s, 2CH), 7.2-7.3 (8H, m, 8CH aromatic), 7.5-7.6 (4H, m, 4CH aromatic), 7.5-7.6 (2H, t, 2CH aromatic), 7.7-7.75 (5H, m, 5CH aromatic), 7.8-7.9 (8H, m, 8CH aromatic), 7.95-8.0 (2H, d, 2CH aromatic); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C 45 (CH_2CO), 64 ($2CH_2O$), 70 (2CH), 92 (2COH), 119 (2CH aromatic), 128 (2CH aromatic), 130 (8CH aromatic), 132 (45CH aromatic), 134 (8CH aromatic), 136 (4CH aromatic), 137 (CBr aromatic), 138 (4CC=O), 147 (2CN aromatic), 170 (4C=O), 190 (C=O) ; HRMS(EI^+) found 831.123 $C_{48}H_{37}BrO_{11}N$ requires 831.123.

2.6. Preparation of Schiff base of 1-[{(methylene)-4-chloro-benzimine}-O-(3`, 4`, 6`-tribenzoyl- β-D-fructofuranosyl]-1H- benzotriazole 8

Equal amounts of nucleoside analogue **5** (2 mmol, 0.4 g) and *p*-chloro aniline (2 mmol, 2 ml) were dissolved in DCM (10 ml) and glacial acetic acid (2 ml). The mixture was heated under reflux for 1.5 h. The mixture was neutralized with $NaHCO_3$ (10 ml) and extracted with $CHCl_3$. The organic layer was washed with water (10 ml) and dried over sodium sulphate. The solvent was evaporated to give the dark brown oil. R_f = 0.75 (benzene: methanol = 6:4); IR (thin film) cm^{-1} 3070 (m) (CH aromatic, stretch), 2925 (m) (CH aliphatic, stretch), 1720 (s) (C=O ester, stretch), 1650 (m) (C=C aromatic, stretch), 1600 (m) C=N imine, stretch), 1512 (m) (N=N, stretch); ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 3.1 (4H, s, $2CH_2OBz$), 3.2 (2H, s, CH_2CN), 3.4 (2H, s, 2CH), 7.2-7.3 (8H, m, 8CH aromatic), 7.5-7.6 (4H, m, 4CH aromatic), 7.5-7.6 (2H, t, 2CH aromatic), 7.7-7.75 (5H, m, 5CH aromatic), 7.8-7.9 (8H, m, 8CH aromatic), 7.95-8.0 (2H, d, 2CH aromatic); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C 45 (CH_2CO), 64 ($2CH_2O$), 70 (2CH), 92 (2COH), 119 (2CH aromatic), 128 (2CH aromatic), 130 (8CH aromatic), 132 (4CH aromatic), 134 (8CH aromatic), 136 (4CH aromatic), 137 (CCl aromatic), 138 (4CC=O), 147 (2CN aromatic), 170 (4*C*=O), 190 (C=O) ; HRMS(EI^+) found 795. 223 $C_{48}H_{38}O_{11}N$ requires 795.223.

2.7. Preparation of Schiff base of 1-[{(methylene)-*p*-methyl-benzimine}-O-(3`, 4`, 6`-tribenzoyl- β-D-fructofuranosyl]-1H- benzotriazole 9

The amounts of nucleoside analogue **5** (2 mmol, 0.4 g) and *p*-toludine (2 mmol, 2 ml) were equally dissolved in DCM (10 ml) and glacial acetic acid (2 ml). The mixture was heated under reflux for 1.5 h. The mixture was neutralized with $NaHCO_3$ (10 ml) and extracted with $CHCl_3$. The organic layer was washed with water (10 ml) and dried over sodium sulphate. The solvent was evaporated to give the dark brown crystal (m.p. 363 K, decomposition); $R_f = 0.75$ (benzene: methanol = 6:4); IR (thin film) cm^{-1} 3070 (m) (CH aromatic, stretch), 2925 (m) (CH aliphatic, stretch), 1720 (s) (C=O ester, stretch), 1650 (m) (C=C aromatic, stretch), 1600 (m) C=N imine, stretch), 1512 (m) (N=N, stretch); ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 2.3 (3H, s, CH_3), 3.1 (4H, s, $2CH_2OBz$), 3.3 (2H, s, CH_2CN), 3.4 (2H, s, 2CH), 7.2-7.3 (8H, m, 8CH aromatic), 7.5-7.6 (4H, m, 4CH aromatic), 7.5-7.6 (2H, t, 2CH aromatic), 7.7-7.75 (5H, m, 5CH aromatic), 7.8-7.9 (8H, m, 8CH aromatic), 7.95-8.0 (2H, d, 2CH aromatic); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C 24 (CH_3), 45 (CH_2CO), 64 ($2CH_2O$), 70 (2CH), 92 (2COH), 119 (2CH aromatic), 128 (2CH aromatic), 130 (8CH aromatic), 132 (5CH aromatic), 134 (8CH aromatic), 136 (4CH aromatic), 137 (C aromatic), 138 (4CC=O), 147 (2CN aromatic), 170 (4C=O), 190 (C=O) ; HRMS(EI^+) found 795. 223 $C_{48}H_{38}O_{11}N$ requires 795.223.

2.8. Preparation of Schiff base of 1-[{(methylene)-3-hydroxy propyl imine}-O-(3`, 4`, 6`-tribenzoyl- β-D-fructofuranosyl]-1H- benzotriazole 10

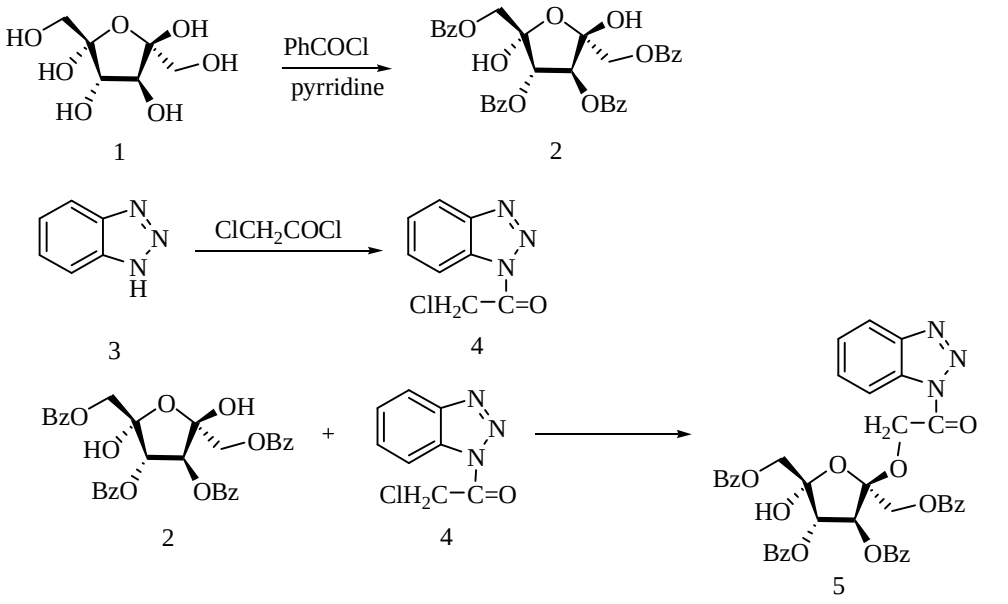
Equal amounts of nucleoside analogue **5** (2 mmol, 0.4 g) and 3-hydroxy propyl amine (2 mmol, 2 ml) were dissolved in DCM (10 ml) and glacial acetic acid (2 ml). The mixture was heated under reflux for 1.5 h. The mixture was neutralized with $NaHCO_3$ (10 ml) and extracted with $CHCl_3$. The organic layer was washed with water (10 ml) and dried over sodium sulphate. The solvent was evaporated to give the dark brown crystal (m.p. 411 K, decomposition); $R_f = 0.75$ (benzene: methanol = 6:4); IR (thin film) cm^{-1} 3320 (s) (OH, stretch), 3070 (m) (CH aromatic, stretch), 2925 (m) (CH aliphatic, stretch), 1720 (s) (C=O ester , stretch), 1650 (m) (C=C aromatic, stretch), 1600 (m) C=N imine, stretch), 1512 (m) (N=N, stretch); ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 2.1-2.3 (1H, s, OH), 3.1 (4H, s, $2CH_2OBz$), 3.2 (2H, s, CH_2CN), 3.5 (2H, s, 2CH), 7.2-7.3 (8H, m, 8CH aromatic), 7.5-7.6 (4H, m, 4CH aromatic), 7.5-7.6 (2H, t, 2CH aromatic), 7.7-7.75 (5H, m, 5CH aromatic), 7.8-7.9 (8H, m, 8CH aromatic), 7.95-8.0 (2H, d, 2CH aromatic); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C 45 (CH_2CO), 64 ($2CH_2O$), 70 (2CH), 92 (2COH), 119 (2CH aromatic), 128 (2CH aromatic), 130 (8CH aromatic), 132 (5CH aromatic), 134 (8CH aromatic), 136 (4CH aromatic), 137 (C aromatic), 138 (4CC=O), 147 (2CN aromatic), 170 (4C=O), 190 (C=O) ; HRMS(EI^+) found 795. 223 $C_{48}H_{38}O_{11}N$ requires 795.223.

3. Results and Discussion

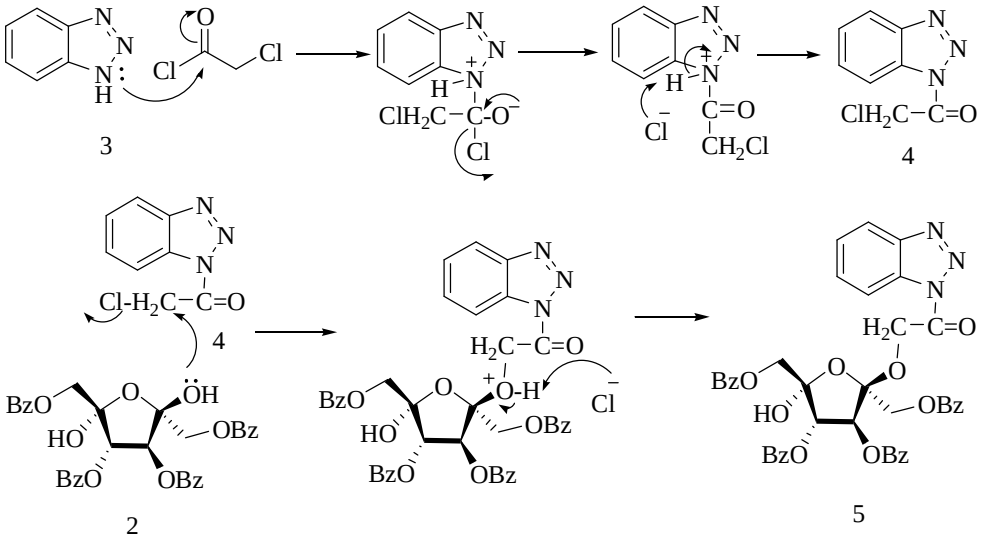
In this research new nucleoside analogues were synthesized using benzotriazole as a nucleobase while D-fructose as a sugar moiety as shown in Scheme 1. In order to obtain fructose as fructofuranose and to protect their hydroxyl as benzoate, this step was achieved by protecting hydroxyls at C-1, C-3, C-4 and C-6 leaving hydroxyl group at C-2 free for further chemical modification [13]. The FT-IR spectrum of **2** showed stretching band at 3230 cm^{-1} (stretch OH), 3064 cm^{-1} (C–H aromatic), 2929 cm^{-1} (CH aliphatic) and at 1720 cm^{-1} (C=O benzoate).

Benztriazole is an important compound, due to the structural similarity of purine. For this reason it was chosen as a nucleobase. In this research chloroacetyl chloride was used to prepare 1-(1-chloroacetyl)-1H-benzotriazole **4** (Scheme 1) [14]. The mechanism of this reaction involves a nucleophilic attack on the carbonyl group of chloroacetyl chloride by the lone pair on the nitrogen atom in the benztriazole (Scheme 2). The second stage of this mechanism occurrs in two steps, in the first, the carbon–oxygen double bond reforms and chloride ion is pushed off. That is followed by removal of a hydrogen ion from the nitrogen of amine to give amide **4**.

IR spectrum of benzotriazole shows stretching band at 3310 cm^{-1} owing to NH group. This band disappears in IR spectrum of 1-(1-chloroacetyl)-1H-benzotriazole **4,** due to generation of amide group in compound **4**. Furthermore, the ^{13}C NMR spectrum of compound **4** demonstrates the signal at 190 ppm belonging to carbon of carbonyl group. Condensation reaction of protected sugar **2** and 1-(1-chloroacetyl)-1H-benzotriazole **4** yields nucleoside analogue **5.** The IR spectra of compound **2** show stretching band of OH at 3250 cm^{-1}. This band disappears in the IR spectrum of nucleoside analogue **5**, due to the formation of ether link between protected sugar and benzotriazol derivative **4**. The ^{1}H NMR spectrum of compound **5** shows characteristic signal as singlet at 3.1, 3.3 and 3.4 ppm belonging to two methylene sugar protons CH_2OBz, protons of CH_2O and methylene CH, respectively.



Scheme 1. Total synthesis of nucleoside analogue **5**

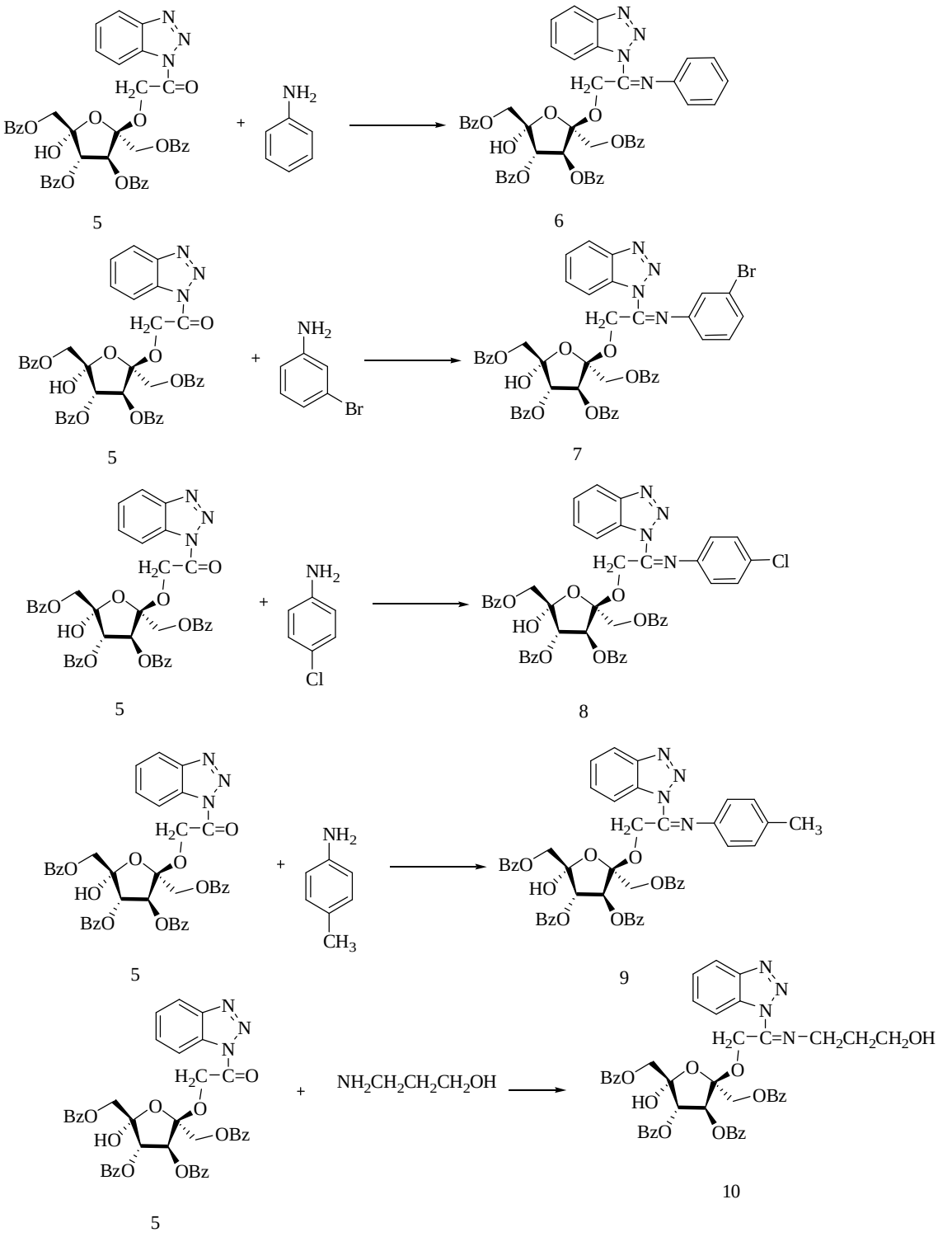


Scheme 2. Full mechanism of condensation reaction of compounds **3** and **4**

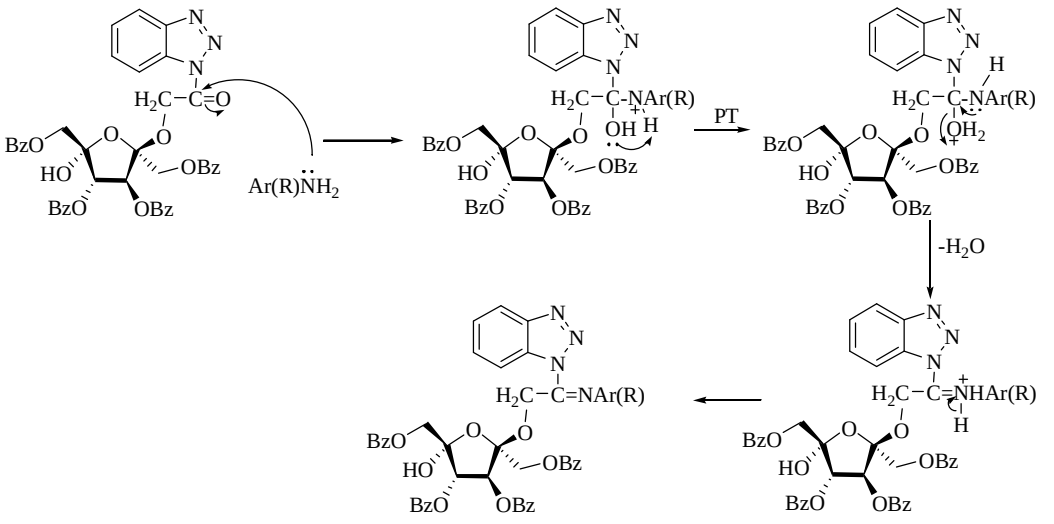
Another step is generating Schiff bases by using aromatic and aliphatic amines (aniline, 3-bromo aniline, 4-chloro aniline, *p*-toludine, and 3-hydroxy propyl amine) as shown in Scheme 3. These amines react with carbonyl group of nucleoside analogue **5** under condensation reaction. The mechanism of formation of Schiff bases starts with nucleophilic attack of amine on carbonyl group of nucleoside analogues to generate iminum ion after eliminated molecule of water, followed by abstraction proton from iminum salt to afford imine group of Schiff base (Scheme 4) [16].

The FT-IR spectrum of nucleoside analogue **5** shows stretching band at 1672 cm^{-1} belonging to carbonyl of amide. This band disappears in the IR spectra of compounds **6-10** and another stretching band at 1600–1620 cm^{-1} owing to C=N imine appeared. Furthermore, the ^{13}C NMR spectra of compounds **6-10** show characteristic signal at 147 ppm belonging to carbon of imine C=N. This signal appears at 190 ppm in the C NMR spectrum of compound **5** belonging to carbonyl C=O.

The ^{1}H NMR spectrum of compound **9** shows signal as singlet at 2.3 ppm belonging to protons of CH_3 in *p*-toludine residue. Moreover, the ^{13}C NMR spectrum of this compound shows signal at 24 ppm owing to carbon of methyl group. ^{1}H NMR spectrum of compound **10** shows broad signal at 2.1 ppm belonging to proton of OH group of 3-hydroxy propyl amine residue.



Scheme 3. Formation of Schiff bases **6-10** from nucleoside analogue **5**



Scheme 4. Mechanism of formation of Schiff bases **6-10**

Table 1

MIC values (inhibition zones in mm) of compounds 5-10

Compounds	Escherichia coli	Pseudomonas auroginosa	Staphylococcus aureus
5	19 ± 1	19 ± 1	18 ± 1
6	19 ± 1	19 ± 1	19 ± 1
7	19 ± 1	19 ± 1	15 ± 1
8	15 ± 1	19 ± 1	15 ± 1
9	19 ± 1	19 ± 1	19 ± 1
10	19 ± 1	19 ± 1	19 ± 1
Chloramphenicol (standard antibacterial)	–	–	19 ± 1

Note: In microbiology, minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that inhibits the visible growth of microorganism after overnight incubation.

3.1. Antimicrobial Activity of Nucleoside Analogues 5-10

The new nucleoside analogues **5-10** were tested *in vitro* for antibacterial activity against Gram positive *staphylococcus aureus*, *pseudomonas auroginosa* and *Escherichia coli* by agar diffusion method. Chlorophenicol and flucanazol were used as control drugs. The observed data on the antimicrobial activity of the compounds and control drugs are given in Table 1. Microdilution broth susceptibility assay was used for the antibacterial evaluation of the compounds and chloramphenicol was used as standard antibacterial agent. Agar plates were surface inoculated uniformly with 100 µl from both cultures of tasted microorganism. The impregnated disks were placed in the middle, and the plates incubated at 278 K for 1 h to permit good diffusion and transferred to an incubator at 310 K for 24 h. The inhibition zones caused by various compounds on the microorganisms were examined; biological activity for prepared compounds is listed in Table 1. The gained results indicate that compounds **7** and **8** show moderate activity against *staphylococcus aureus* while compounds **9** and **10** show good activity against same gram positive bacteria. Most of these compounds show significant activity towards these bacteria. The variance of biological activity refers to different subsistence in tested compounds.

4. Conclusions

D-fructose has been chosen as starting material as first synthon for the synthesis of nucleoside analogues after protected OH groups except OH group at C-2 was left for the chemical modification. The second synthon was produced from reaction of benzatriazole and chloroacetyl chloride. Imine group C=N was introduced to new nucleoside analogues by condensation reaction of carbonyl group of nucleoside analogues and different amines to afford new Schiff bases. Study of biological activity of these new nucleoside analogues shows that most of these analogues exhibit higher degree of activity,

due to the possible activity of these compounds against HIV (Human Immune Deficiency Virus); a preliminary test should be conducted.

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СИНТЕЗ, ХАРАКТЕРИСТИКА ТА АНТИМІКРОБНА АКТИВНІСТЬ НОВИХ НУКЛЕОЗИДНИХ АНАЛОГІВ З БЕНЗОТРИАЗОЛУ

Анотація: *Синтезовано і охарактеризовано нові похідні 1-('1,' 3, '4,' 6-тетрабензоїл-β-D-фруктофураносил)-1H-бензотриазолу та 1-('1,' 3, '4,' 6-тетрабензоїл-β-D-фруктофураносил)-1H-бензотриазолу з основами Шиффа. З використанням бензоїлхлориду синтезовано захищену D-фруктозу, з подальшим нуклеофільним приєднанням/елімінацією між бензотриазолом і хлорацетил хлоридом для одержання 1-(1-хлорацетил)-1H-бензотриазолу. Одержано новий аналог нуклеозидів реакцію конденсації захищеної фруктози і 1-(1-хлорацетил)-1H-бензотриазолу. Нові аналоги нуклеозидів піддавалися другій реакції конденсації з різними ароматичними і аліфатичними амінами, для одержання нових основ Шиффа. Проведено дослідження одержаних аналогів за допомогою Фур'є-спектроскопії, ^{1}H ЯМР, ^{13}C ЯМР, емісійної спектроскопії. Аналоги протестовані на вплив різних бактерій для оцінки їх як антибактеріальних засобів.*

Ключові слова: *антибактеріальні засоби, бензотриазол, аналоги нуклеозидів, захищена фруктоза, основи Шиффа.*