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A novel synthesis of acyclonucleosides via allylation of 3-[1-(phenylhydrazono)-L-*threo*-2,3,4-trihydroxybut-1-yl]quinoxalin-2(1*H*)one

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Dedicated to Professor Hassan S. El Khadem on the occasion of his 80th birthday and to Professor El Sayed H. El Ashry on his 60th birthday

Abstract

The allylation of 3-[1-(phenylhydrazono)-L-*threo*-2,3,4-trihydroxybut-1-yl]quinoxalin-2(1*H*)one (**1**) gave, in addition to the anticipated 1-*N*-allyl derivative (**2**), a dehydrative cyclized product, 1-*N*-allyl-3-[5-(hydroxymethyl)-1-phenylpyrazol-3-yl]quinoxalin-2-one (**4**) and its isomeric *O*-allyl derivative **3**. The *O*-allyl group in **3** underwent acetolysis under acetylation conditions, in addition to the acetylation of the hydroxyl group, to afford 2-acetoxy-3-[5-(acetoxymethyl)-1-phenylpyrazol-3-yl]quinoxaline (**8**) instead of the *O*-acetyl derivative of **3**. Allylation of the tri-*O*-acetyl derivative of **1** caused the elimination of a molecule of acetic acid in addition to N-allylation to give 1-*N*-allyl-3-[3,4-di-*O*-acetyl-2-deoxy-1-(phenylhydrazono)but-2-en-1-yl]quinoxalin-2-one (**11**). Hydroxylation of the allyl group gave a glycerol-1-yl acyclonucleoside which can be alternatively obtained by a displacement reaction of the tosyloxy group in 2,3-*O*-isopropylidene-1-*O*-(*p*-tolylsulfonyl)glycerol (**14**), followed by deisopropylidenation. 1-*N*-(2,3-Dibromopropyl)-3-[5-(hydroxymethyl)-1-(4-bromophenyl)pyrazol-3-yl]quinoxalin-2-one (**15**) underwent azidolysis to give a 2,3-diazido derivative. The assigned structures were based on spectral analysis. The activity of compounds **2**, **4**, **6**, and **15** against hepatitis B virus was studied.

Keywords: Allyl bromide; NaH/DMF; Pyrazolylquinoxaline; Acyclonucleosides; Allylation

1. Introduction

Various pharmacological properties such as hypnotic,[1] antiinflammatory,[2] analgesic,[3] tranquilizing, antidepressant,[4] fungicidal,[5] and anthelmintic[6] effects have been reported for quinoxalin-2-ones. Similarily, pyrazole derivatives have various biological activities.[7–14] The biological activities of acyclonucleosides, as a consequence of the potent activity reported for some of their members after the discovery of acyclovir, has led to synthesis of a diversity of structures of either the lead compounds or their analogues, with many variations in order to enhance biological activity and selectivity, or to lower their toxicity.[15–29]

Consequently, the foregoing aspects have attracted our attention towards the synthesis of acyclonucleoside analogues carrying a pyrazolylquinoxaline moiety as a base, in order to have a combination of quinoxaline and pyrazole rings with an acyclic side-chain. This investigation has shown a new transformation from an acyclic C-nucleoside of the seco type, 3-[1-(phenylhydrazono)-L-*threo*-2,3,4-trihydroxybut-1-yl]quinoxalin-2(1*H*)one, to an acyclic *N*-nucleosides of the tetra seco type, a classification recently used for acyclonucleosides.[20–22]

2. Results and discussion

The starting material 3-[1-(phenylhydrazono)-L-*threo*-2,3,4-trihydroxybut-1-yl]quinoxalin-2-(1*H*)one (**1**) was prepared as reported in the literature.[25] Attempted allylation of **1** with allyl bromide in the presence of

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potassium carbonate in *N*,*N*-dimethylformamide gave a mixture of three products (**2**–**4**). Structural elucidation of these products indicated that compound **2** is the expected *N*-allylated derivative, isolated as an orange crystalline product, 1-*N*-allyl-3-[1-(phenylhydrazono)-L-*threo*-2,3,4-trihydroxybut-1-yl]quinoxalin-2-one (**2**). Elemental analysis of **2** agreed with the molecular formula $C_{21}H_{22}N_4O_4$. Its IR spectrum showed bands at 1648 and 3403 cm^{-1} for the OCN and OH groups, respectively. The ^1H NMR spectrum showed two doublets of doublets at δ 3.53 and 3.90 due to H-4,4′, a doublet at δ 4.15 due to H-2, and a multiplet at δ 4.97 due to H-3 of the side-chain, confirming the presence of the trihydroxybutyl group. The presence of one allyl group was indicated by the presence of a doublet (δ 4.78) and two multiplets (δ 5.30 and 6.47). Moreover, the phenylhydrazone residue remained unaffected, as the spectrum showed the presence of a singlet at δ 9.77 due to NH of the hydrazone moiety.

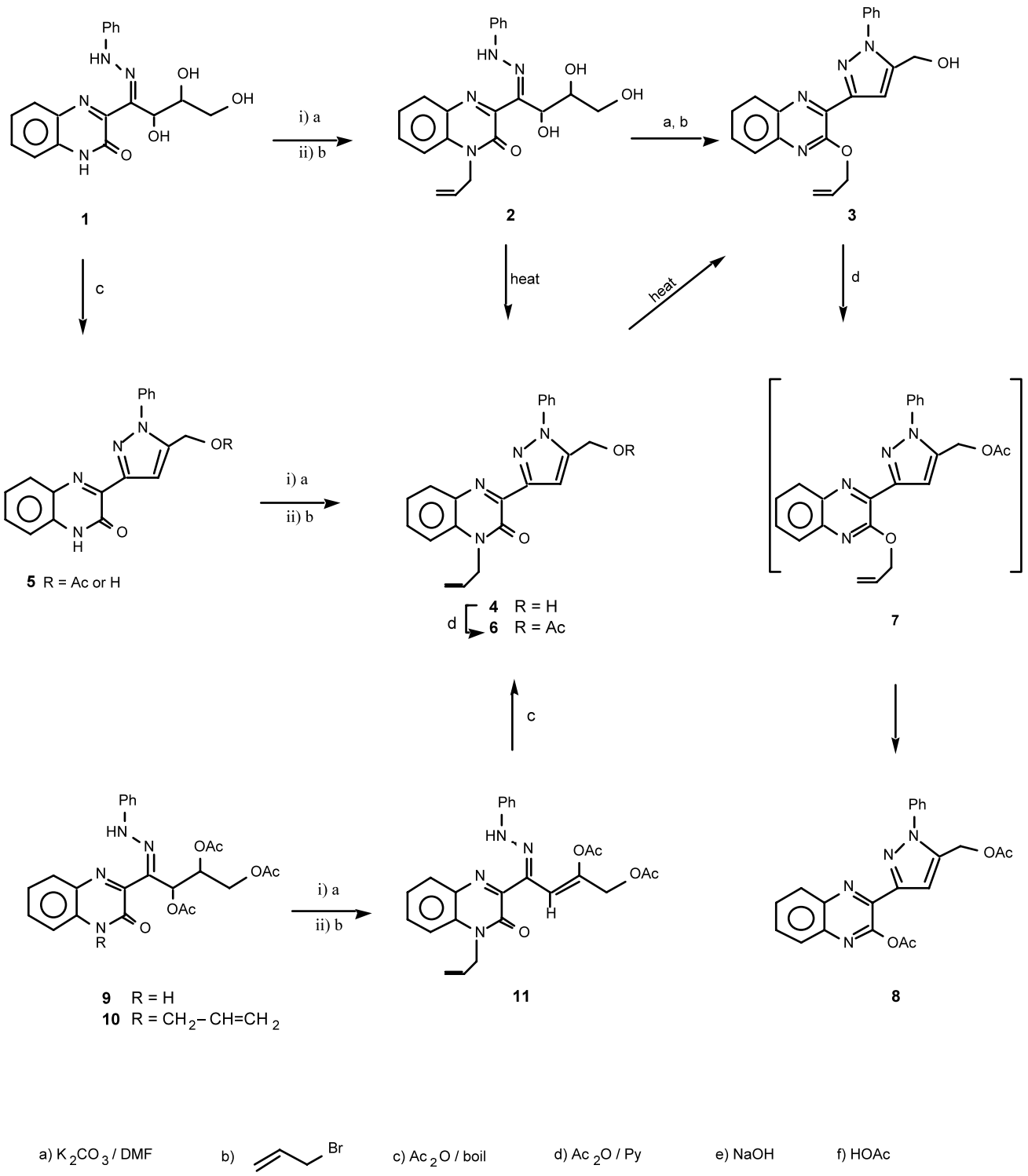
The second product was assigned the structure **4** based on the following data. It showed the presence of a band in its IR spectrum at 1645 cm^{-1}, indicating the presence of an OCN group. Its ^1H NMR spectrum showed the presence of one hydroxyl group giving a broad singlet at δ 3.00 and a methylene group as a singlet at δ 4.51. This indicated that a dehydrative cyclization of the glycerol-1-yl moiety and the hydrazone residue in the quinoxalinone **2**, during the allylation, had taken place and the allyl group was introduced on the nitrogen of the quinoxalinone ring, as indicated from the ^1H NMR spectrum which showed as a doublet at δ 4.81 and two multiplets at δ 5.18 and 5.63. Also the elemental analysis agreed with the molecular formula $C_{21}H_{18}N_4O_2$. The structure of **4** was chemically confirmed by an unequivocal synthesis of its acetyl derivative by the allylation of 3-[5-(acetoxymethyl)-1-phenylpyrazol-3-yl]quinoxalin-2(1*H*)one (**5**)[25] (R = Ac) with allyl bromide in presence of K_2CO_3 in DMF to give 1-*N*-allyl-3-[5-(acetoxymethyl)-1-phenylpyrazol-3-yl]quinoxalin-2-one (**6**), which was found to be identical with the product of acetylation of **4**. This confirmed the basic skeleton of the product as a pyrazolyl quinoxalinone derivative. Its IR spectrum showed, in addition to the band of the OCN group, a band at 1730 cm^{-1} for the acetyl group. Its ^1H NMR spectrum confirmed the presence of a CH_2OAc group and one allyl group.-Schemes 1 and 2.

The third product, **3**, showed no absorption in the range 1660 cm^{-1}, indicating absence of the OCN group. Its ^1H NMR spectrum showed a doublet at δ 4.42 and two multiplets at δ 5.20 and 5.90 characteristic for the presence of one allyl group. The spectrum indicated the absence of the glycerol-1-yl side-chain and showed instead a doublet at δ 5.00 and a triplet at δ 5.56; upon deuteration the former doublet was transformed into a singlet and the triplet disappeared, indicating the presence of an CH_2OH group. The elemental analysis agreed with the molecular formula $C_{21}H_{18}N_4O_2$, which upon combination with the spectral data, led to the conclusion that the allyl group had been introduced on the oxygen atom of the quinoxalinone ring, and the trihydroxybutyl hydrazone residue had undergone dehydrative cyclization by losing two molecules of water. The presence of the allyl group on oxygen rather than on the nitrogen of the quinoxalinone can be explained as being due to rearrangement of the *N*-allyl group via a Claisen rearrangement to give the *O*-allyl derivative.

Attempted acetylation of compound **3** by Ac_2O–pyridine did not afford the expected product **7**. Its IR spectrum showed no absorption for an amide group and showed a band at 1740 cm^{-1}. Its ^1H NMR spectrum showed a methylene group, which appeared downfield (δ 5.23) as a singlet. However, no signals were present corresponding to the allyl group, but an extra acetyl group appeared, with two singlets at δ 1.57 and 2.12. Moreover, deacetylation of **8** gave **5** (R = H). These data led to assignment of structure **8** for this product. The presence of the acetoxy group in place of the allyloxy group would result from an acetolysis process.

Acetylation of compound **1** afforded **9**,[14] whose allylation with allyl bromide in presence of K_2CO_3 in DMF gave a product whose spectral data ruled out the expected structure 1-*N*-allyl-3-[L-*threo*-2,3,4-triacetoxy-1-(phenylhydrazono)but-1-yl]quinoxalin-2-one (**10**) or even a product resulting from the eliminative cyclization process, such as **6**. Its IR spectrum showed acetoxy and amide carbonyl absorption bands at 1748, 1733, and 1645 cm^{-1}, respectively. These data as well as its ^1H NMR spectrum established its acyclic nature. The two signals at δ 2.08 and 2.19 were assigned as acetyl groups and two singlets at δ 5.17 (2H) and 6.23 (1H) were attributed, respectively to methylene protons and a methine proton, each without coupling. In addition, the methine proton appeared at a lower field indicating its linkage to a double bond. Moreover, the expected signals for the allyl, aromatic, and NH protons were present. These data indicated that, under the conditions of allylation, an elimination of one molecule of acetic acid from the side-chain of the expected product **10** had taken place, to give 1-*N*-allyl-3-[3,4-di-*O*-acetyl-2-deoxy-1-(phenylhydrazono)but-2-en-1-yl]quinoxalin-2-one (**11**).

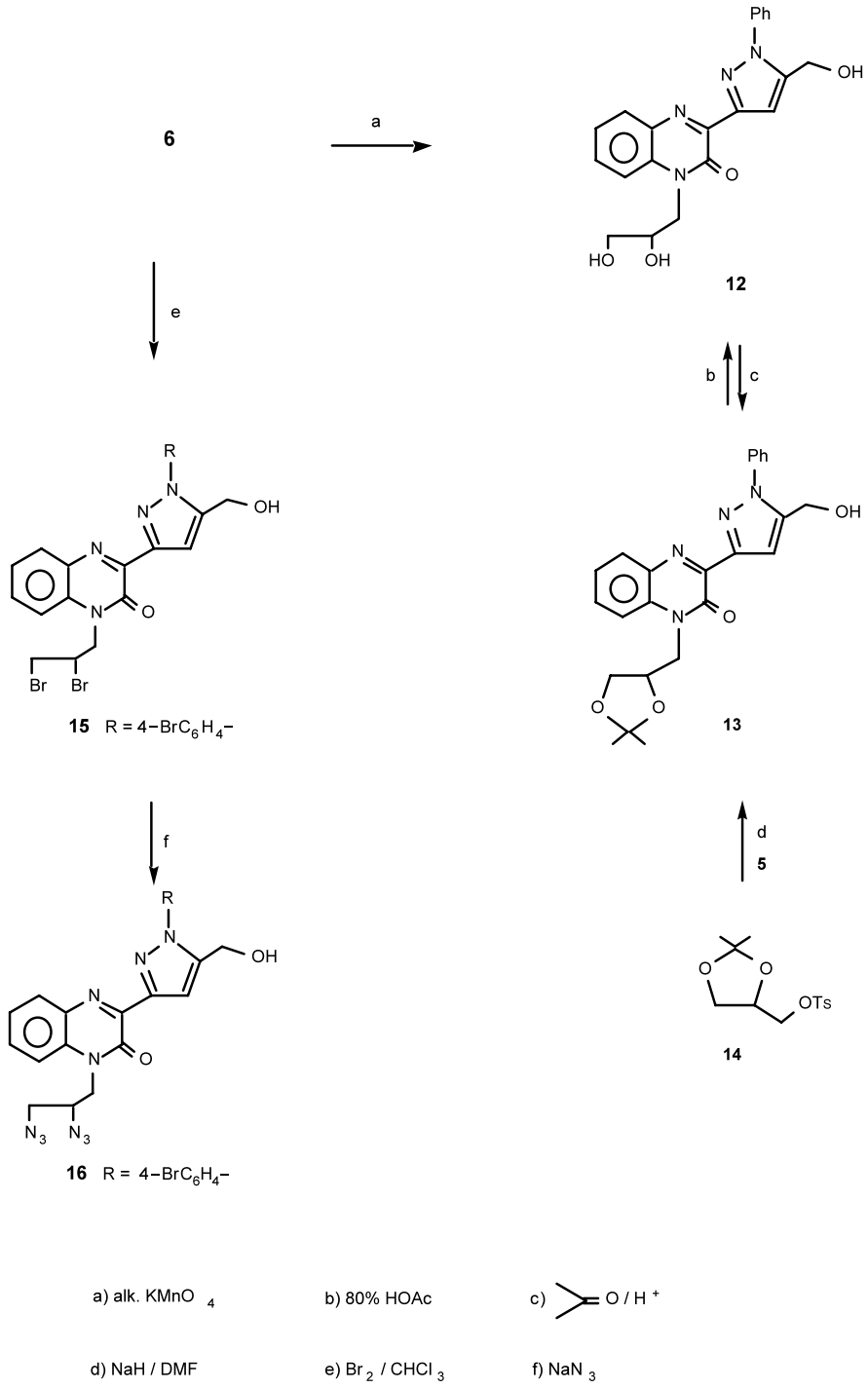
The allyl group is an excellent precursor for various chemical modifications. Thus, the reaction of **6** with bromine in $CHCl_3$ gave a product whose combustion analysis and mass spectrum indicated the presence of three bromine atoms. Its IR spectrum showed, in addition to the amide band, a band at 3425 cm^{-1} due to hydroxyl group; the acetyl band of **6** was absent. This indicated that a deacetylation process had taken place during the bromination. Its ^1H NMR spectrum showed the presence of the CH_2O signal of the starting material



Scheme 1.

at δ 4.64. The two doublets of doublets, at δ 3.48, and 3.61, the multiplet at δ 4.17, and the doublet at δ 4.35 indicated the addition of bromine to the double bond, along with bromination of the phenyl group. The introduction of an azide group in nucleoside analogues is of potential significance from the biological point of view. Thus, nucleophilic displacement of the bromine in compound **15** by sodium azide afforded the diazide **16**. Its IR spectrum showed the azide band at 2103 cm^{-1}.

Another type of acyclonucleoside of the tetra seco type, possessing a glycerol-1-yl side-chain was prepared. Hydroxylation of compound **6** with alkaline $KMnO_4$



Scheme 2.

afforded compound **12**, which could alternatively be obtained by treatment of compound **5** (R = Ac) with NaH in dry DMF followed by 2,3-*O*-isopropylidene-1-*O*-(*p*-tolylsulfonyl)-glycerol (**14**)[30] to give **13**, deprotection of which with 80% acetic acid at reflux temperature gave 1-(2,3-dihydroxypropyl)-3-[5-(hydroxymethyl)-1-phenylpyrazol-3-yl]quinoxalin-2-one (**12**), whose reaction with acetone in sulfuric acid gave back compound **13**. The structures were confirmed from their spectral data. The IR spectrum of **13** showed a band at 3411 (OH) and a band at 1664 cm^{-1} (OCN). Its ^1H NMR showed two singlets at δ 1.36 and 1.42 for the two methyl groups of the isopropylidene groups, whose $\Delta\delta$ was 0.06, in agreement with a terminal isopropylidene group according to the shift rule of El Ashry.[31,32]

Viral screening against HBV of selected compounds indicated that compound **15** was the most effective one in this series against HBV, with a selective index >

476.2. This compound showed high inhibition with high cytotoxicity, while compounds **2** and **6** showed moderate inhibition of viral replication and mild cytotoxicity, with selective index as >154 and >303, respectively. The effective concentration of compound **4** was 10.0 μM, which showed low inhibition and low cytotoxicity with the selective index >91 (Tables 1 and 2)

3. Experimental

3.1. General methods

Melting points were determined with a Mel-Temp apparatus and are uncorrected. IR spectra were recorded for the compounds in a KBr matrix with a Unicam SP 1025 spectrophotometer. ^{1}H NMR spectra were recorded on EM-390 and a Bruker AC 300 MHz spectrometers using Me_4Si as the standard. Chemical shifts are given on the δ scale. Mass spectra were recorded using electron ionization (EI) on a Jeol JMS AX-500 spectrometer. Thin layer chromatography (TLC) was performed on Baker-Flex silica gel IB-F plates using 2:3 EtOAc–hexane as developing solvent. The spots were detected by their characteristic color and by UV absorption. The biological testing was carried out at the Liver Institute, Menoufia University, Egypt. Microanalyses were performed at the microanalytical laboratory at the Faculty of Science, Cairo University.

3.2. Reaction of 3-[1-(phenylhydrazono)-L-*threo*-2,3,4-trihydroxybut-1-yl]quinoxalin-2(1*H*)one with allyl bromide

A solution of 3-[1-(phenylhydrazono)-L-*threo*-2,3,4-trihydroxybut-1-yl]quinoxalin-2(1*H*)one (**1**) (1.77 g, 5 mmol) in dry DMF (10 mL) was treated with anhyd K_2CO_3 (1.38 g, 10 mmol) and the mixture was heated at 80 °C for 2 h. The resulting mixture was cooled, allyl bromide (1.2 mL, 10 mmol) was added, and the mixture was stirred for 4 h at room temperature (rt). The mixture was diluted with water and the product collected by filtration, washed with water and EtOH. TLC showed the presence of three products. The crude product was purified by fractional crystallization from absolute EtOH to give, the following 3 products.

Table 1
Cytotoxic effect (CC_{50}), inhibitory concentration (IC_{50}) and selective index (SI) of selected compounds

Compound	HBV DNA IC_{50} (μM)	Hep G2 2.2.15 CC_{50} (μM)	SI
Lamivudine	<0.1	>100	>1000
2	0.65	>100	>153.9
4	1.10	>100	>90.9
6	0.33	>100	>303.0
15	0.21	>100	>476.2

3.3. 1-*N*-Allyl-3-[1-(phenylhydrazono)-L-*threo*-2,3,4-trihydroxybut-1-yl]quinoxalin-2-one (2)

Orange crystals, R_f 0.184, yield 0.7 g (36%), mp 152–154 °C; IR (KBr) 3403 (OH), 1648 (OCN) and 1600 cm^{-1} (C=N and C=C); ^{1}H NMR (Me_2SO-d_6): δ 3.53, 3.90 (2 dd, 2 H, $J_{2,3'}$ 6.0, $J_{2,3}$ 4, $J_{3,3'}$ 12 Hz, H-4,4′); 4.15 (d, 1 H, J 4.5 Hz, H-2); 4.78 (d, 2 H, J 7.5 Hz, N–CH_2); 4.97 (m, 1 H, H-3); 5.30 (m, 2 H, =CH_2); 6.47 (m, 1 H, =CH); 6.79–7.63 (m, 9 H, Ar–H) and 9.77 (s, 1 H, D_2O exchangeable, NH). Anal. Calcd for $C_{21}H_{22}N_4O_4$ (394.42): C, 63.95; H, 5.62; N, 14.21. Found: C, 63.72; H, 5.50; N, 14.0.

3.4. 1-*N*-Allyl-3-[5-(hydroxymethyl)-1-phenylpyrazol-3-yl]quinoxalin-2-one (4)

Pale yellow crystals R_f 0.553, yield 0.8 g (41%), mp 176–178 °C; IR (KBr) 3310 (OH); 1642 (OCN) and 1600 cm^{-1} (C=N and C=C); ^{1}H NMR ($CDCl_3$): δ 3.00 (bs, 1 H, OH); 4.51 (s, 2 H, CH_2OH); 4.81 (d, 2 H, J 7.5 Hz, N–CH_2); 5.18 (m, 2 H, =CH_2); 5.63 (m, 1 H, =CH); 7.13–7.59, 7.89 (m, d, 10 H, Ar–H + pyrazole proton). Anal. Calcd for $C_{21}H_{18}N_4O_2$ (358.38): C, 70.38; H, 5.06; N, 15.63. Found: C, 70.50; H, 4.90; N, 15.50.

3.5. 2-*O*-Allyl-3-[5-(hydroxymethyl)-1-phenylpyrazol-3-yl]quinoxaline (3)

Pale yellow product, R_f 0.58, yield 0.4 g (20%), mp 105–108 °C; IR (KBr) 3414 (OH) and 1623 cm^{-1} (C=N and C=C); ^{1}H NMR (Me_2SO-d_6): δ 4.42 (d, 2 H, J 9.0 Hz, O–CH_2); 5.00 (s, 2 H, CH_2OH); 5.20 (m, 2 H, =CH_2); 5.56 (t, 1 H, OH); 5.90 (m, 1 H, =*CH*); 7.13–7.59, 7.89 (m, d, 10 H, Ar–H, pyrazole proton). Anal. Calcd for $C_{21}H_{18}N_4O_2$ (358.38): C, 70.38; H, 5.06; N, 15.63. Found: C, 70.20; H, 5.00; N, 15.70.

3.6. 1-*N*-Allyl-3-[5-(acetoxymethyl)-1-phenylpyrazol-3-yl]quinoxalin-2-one (6)

3.6.1. Method (a). A cold solution of 1-*N*-allyl-3-[5-hydroxymethyl)-1-phenylpyrazol-3-yl]quinoxalin-2-one (**4**) (0.3 g, 0.84 mmol) in dry pyridine (5 mL) was treated with Ac_2O (5 mL). The mixture was kept overnight at rt, diluted with ice-cold water, and the product was filtered off, washed repeatedly with water, and dried, yield 0.24 g (71%). The crude product was recrystallized from

Table 2
Results of inhibition of HBV replication by selected compounds

Compound	Concentration (μM)	HBV DNA in supernatant (pg/mL)	Hep G_2 viable cells
Lamivudine	1.0	0.25	1.03
	10.0	0.18	1.01
	100.0	0.15	1.07
2	1.0	0.41	0.04
	10.0	0.36	0.03
	100.0	0.27	0.03
4	1.0	0.16	0.02
	10.0	0.13	0.01
	100.0	0.10	0.01
6	1.0	0.25	0.05
	10.0	0.24	0.04
	100.0	0.18	0.03
15	1.0	0.75	0.81
	10.0	0.61	0.70
	100.0	0.40	0.61

A value of 1.0 pg HBV DNA /mL corresponds to approximately 3×10^3 HBV virions/mL.

EtOH to give colorless needles; mp 195–196 °C; IR (KBr) 1730 (OAc) and 1640 cm^{-1} (OCN); ^1H NMR ($CDCl_3$): δ 2.03 (s, 3 H, CH_3CO); 4.96 (d, 2 H, J 7.5 Hz, N–CH_2); 5.06 (s, 2 H, CH_2OAc); 5.23 (m, 2 H, =CH_2); 5.89 (m, 1 H, =CH); 7.16–7.57, 7.92 (m, d, 10 H, Ar–H + pyrazole–H). Anal. Calcd for $C_{23}H_{20}N_4O_3$ (400.42): C, 68.99; H, 5.04; N, 13.99. Found: C, 69.00; H, 5.00; N, 13.80.

3.6.2. Method (b). A stirred solution of 3-[5-(acetoxymethyl)-1-phenylpyrazol-3-yl]quinoxalin-2-(1*H*)-one (**5**) (R = Ac)[25] (0.40 g; 1.1 mmol) in dry DMF (5 mL) was treated with anhyd K_2CO_3 (0.15 g; 1.1 mmol) and allyl bromide (0.30 mL; 2.5 mmol) at rt for 4 h. The mixture was diluted with water and the product collected by filtration and crystallized from EtOH as colorless needles, yield 0.3 g (68%), mp 195–196 °C; IR and ^1H NMR spectra were superimposable with the spectra of the product from method (a).

3.7. 1-*N*-Allyl-3-[5-(hydroxymethyl)-1-phenylpyrazol-3-yl]quinoxalin-2-one (4)

A solution of **5** (R = Ac) (0.36 g, 1.0 mmol) and NaH (0.024 g, 1.0 mmol) in dry DMF (10 mL) was stirred at rt for 2 h. Then allyl bromide (0.30 mL, 2.5 mmol) was added and the mixture was stirred for 4 h. The mixture was diluted with water, the product obtained was filtered off, washed with water, and dried. It was crystallized from EtOH as pale yellow crystals, yield 0.30 g (84%). This compound was identical to that obtained from the reaction of **1** with allyl bromide in K_2CO_3–DMF.

3.8. 2-Acetoxy-3-[5-(acetoxymethyl)-1-phenylpyrazol-3-yl]quinoxaline (8)

A solution of **3** (0.2 g, 0.56 mmol) in pyridine (5 mL) was cooled in an ice-bath, then treated with Ac_2O (5 mL) with stirring. The mixture was kept at rt overnight, then poured onto crushed ice. The product that separated, was filtered off, washed with water, and dried. It was crystallized from EtOH as colorless crystals, yield 0.2 g (89%), mp 200–203 °C; IR (KBr) 1740 cm^{-1} (OAc); ^1H NMR ($CDCl_3$): δ 1.57, 2.12 (2s, 6 H, 2 CH_3CO); 5.23 (s, 2 H, CH_2OAc); 7.51–7.89, 8.03, 8.42 and 8.64 (m, s, 2 d, 10 H, Ar–H, pyrazole–H). Anal. Calcd for $C_{22}H_{18}N_4O_4$ (402.39): C, 65.66; H, 4.50; N, 13.92. Found: C, 65.45; H, 4.30; N, 14.00.

3.9. 2-Hydroxy-3-[5-(hydroxymethyl)-1-phenylpyrazol-3-yl]quinoxaline (5)(R = H)

A solution of **8** (0.4, 1.0 mmol) in EtOH (10 mL) was treated with 1M NaOH solution, (10 mL). The mixture was heated under reflux for 4 h. The clear solution obtained was neutralized with AcOH and left to cool, a colorless product separated out. The product was filtered off, washed with water, and crystallized from EtOH, mp 251 °C [Lit.[25] 250–251 °C].

3.10. 1-*N*-Allyl-3-[3,4-di-*O*-acetyl-2-deoxy-1-(phenylhydrazono)but-2-en-1-yl]quinoxalin-2-one (11)

A solution of **9**[14] (0.5 g, 1.0 mmol) in dry DMF (5 mL) was treated with anhyd K_2CO_3 (0.28 g, 2.0 mmol) and allyl bromide (0.24 mL, 2.0 mmol) and processed as

before to give **11**, yield 0.3 g (63%), mp 144–146 °C; IR (KBr) 1748, 1733 (OAc); 1645 (OCN) and 1599 cm^{-1} (C=N); ^1H NMR ($CDCl_3$): δ 2.08, 2.19 (2s, 6 H, 2 CH_3CO); 4.42, 4.91 (dd, 2 H, *J* 1.5, 3.6 Hz, N–CH_2); 5.17 (s, 2 H, CH_2OAc); 5.23 (m, 2 H, =CH_2); 5.97 (m, 1 H, =CH); 6.23 (s, 1 H, CHOAc); 7.24–7.80 (m, 9 H, Ar–H) and 9.13 (s, 1 H, D_2O exchangeable NH). Anal. Calcd for $C_{25}H_{24}N_4O_5$ (460.47): C, 65.21; H, 5.25; N, 12.17. Found: C, 65.00; H, 5.10; N, 11.9.

3.10.1. Effect of boiling acetic anhydride on compound 11. A suspension of compound **11** (0.1 g, 0.2 mmol) in Ac_2O (10 mL) was heated under reflux. The reflux was continued for 30 min after the solid had dissolved. The resulting solution was cooled and then poured into ice–water. The product obtained was filtered off, washed with water, and dried. It was crystallized from EtOH to give product identical with compound **6** (0.05 g, 57%): mp 194–196 °C.

3.11. 1-(2,3-Dihydroxypropyl)-3-[5-(hydroxymethyl)-1-phenylpyrazol-3-yl]quinoxalin-2-one (12)

3.11.1. Method (a). A suspension of compound **6** (0.2 g, 0.5 mmol) in a solution of Na_2CO_3 (0.1 g, 0.9 mmol) in H_2O (5 mL) was stirred at rt. Alkaline $KMnO_4$ was then added dropwise, until the color of $KMnO_4$ persisted. The mixture was stirred for 2 h and then kept overnight. The product that separated out was filtered off, washed with water and crystallized from EtOH as colorless crystals, yield 0.1 g (51%), mp 97–98 °C; IR (KBr) 3439 (OH); 1640 (OCN) and 1600 cm^{-1} (C=N); ^1H NMR (Me_2SO-d_6–D_2O): δ 4.08 (dd, 1 H, *J* 5.4, 12.6 Hz; H-3′a); 4.15 (dd, 1 H, *J* 5.1 Hz, H-3′b); 4.41 (m, 2 H, H-1′a, H-2′); 4.57 (d, 1 H, *J* 10.2 Hz, H-1′b); 5.15 (s, 2 H, CH_2OH); 7.37–7.86 and 7.88 (m, d, 10 H, Ar–H+ Pyrazole–H). Anal. Calcd for $C_{21}H_{20}N_4O_4$ (392.4): C, 64.27; H, 5.14; N, 14.28. Found: C, 64.00; H, 4.90; N, 14.00.

3.11.2. Method (b). Compound **13** (0.2 g, 0.46 mmol) was refluxed in 80% AcOH (10 mL) for 2 h the solvent was evaporated under diminished pressure to give a colorless product, yield 0.12 g (67%), mp 96–98 °C. This product was identical with the product from method a.

3.12. 3-[5-(Hydroxymethyl)-1-(2,3-*O*-isopropylidenedioxypropyl-1-phenylpyrazol-3-yl]quinoxaline-2-one (13)

3.12.1. Method (a). A stirred solution of **5** (R=Ac) (2.0 g, 5.6 mmol) in anhyd DMF (10 mL) was added NaH (0.14 g, 6.0 mmol) and after evolution of H_2, was almost complete the mixture was heated to 100 °C for 1 h. Then compound **14**[30] (1.52 g, 6.0 mmol) was added, and the mixture was stirred for additional 8–10 h at 100 °C, and cooled to rt, whereupon a white product separated out. It was filtered off, washed with EtOH, and recrystallized from EtOH to give colorless crystals of **13**, yield 1.5 g (63%), mp 190–192 °C; IR (KBr) 3411 (OH) and 1664 cm^{-1} (OCN); ^1H NMR ($CDCl_3$): δ 1.36, 1.42 (2s, 6 H, $2CH_3$); 1.67 (bs, 1 H, D_2O-exchangable OH); 3.57 (m, 2 H, H-1′a, H-3′a); 3.65 (dd, 1 H, *J* 2.8, 6.6 Hz, H-1′b); 4.01 (m, 1 H, H-3′b); 4.35 (m, 1 H, H-2′); 4.73 (d, 2 H, *J* 29.0 Hz, CH_2OH) and 7.26–8.08 (m, 10 H, Ar–H+ pyrazole–H). Anal. Calcd for $C_{24}H_{24}N_4O_4$ (432.46): C, 66.65; H, 5.60; N, 12.96. Found: C, 66.40; H, 5.50; N, 13.10.

3.12.2. Method (b). Compound **12** (0.1 g, 0.25 mmol) was stirred vigorously with dry acetone (10 mL) and 96% H_2SO_4 (3 drops) for 2 h, and then kept overnight at rt. The resulting mixture was neutralized by the addition of solid anhyd Na_2CO_3, and filtered, and the inorganic salts were well washed with dry acetone. The acetone solution was evaporated in vacuo at 30–40 °C, and the resulting product dissolved in EtOH, the product that separated out was filtered off, and dried, yield 0.12 g (51%). This product was identical with the product from Method (a).

3.13. 1-*N*-(2,3-Dibromopropyl)-3-[5-(hydroxymethyl)-1-(4-bromophenyl)pyrazol-3-yl]quinoxaline-2-one (15)

To suspension of **6** (0.8 g, 2.0 mmol) in $CHCl_3$ (10 mL) was added dropwise bromine (2 mL), during 2 h with occasional shaking. The mixture was kept overnight at rt diluted with water, whereupon a yellow product separated out. It was filtered off, washed with water, dried, and crystallized from EtOH to give a yellow crystals of **15**, yield 0.9 g (76%), mp 118–120 °C; IR (KBr) 3425 (OH); 1658 (OCN) and 1597 cm^{-1} (C=N); ^1H NMR (Me_2SO-d_6): δ 3.48, and 3.61 (2dd, 2 H, *J* 5.4, 6.0, 10.2, 21.9 Hz, CH_2Br); 4.17 (m, 1 H, CHBr); 4.35 (d, 2 H, *J* 6.6 Hz, N–CH_2); 4.64 (s, 2 H, CH_2OH); 4.90 (s, 1 H, D_2O exchangeable, OH); 7.42–7.92 and 8.22 (m, d, 9 H, Ar–H and pyrazole–H). Anal. Calcd for $C_{21}H_{17}Br_3N_4O_2$ (597.13): C, 42.24; H, 2.87; N, 9.38. Found: C, 41.90; H, 2.50; N, 9.00.

3.14. 1-*N*-(2,3-Diazidopropyl)-3-[5-(hydroxymethyl)-1-(4-bromophenyl)pyrazol-3-yl]quinoxaline (16)

A mixture of **15** (0.5 g, 0.84 mmol) and NaN_3 (0.13 g, 2.0 mmol) in dry DMF (5 mL) was heated for 3 h at 80 °C. The solvent was evaporated in vacuo, and the resulting product was dissolved in EtOH, decolorized with charcoal, and concentrated. The product yield 0.3 g (68%) that separated out was filtered off and recrystallized from EtOH to give pale yellow crystals, mp 130–132 °C; IR (KBr) 3389 (OH); 2103 (azide) and 1646 cm^{-1} (OCN); ^1H NMR ($CDCl_3$): δ 3.46 (dd, 1 H, *J* 1.8,

9.9 Hz, H- 3′a); 3.49 (dd, 1 H, *J* 8.1 Hz, H-3′b); 4.27 (bs, 1 H, H-1′a); 4.69 (m, 2 H, H-1′b, H-2′); 4.71 (s, 2 H, CH_2OH); 5.20 (bs, 1 H, D_2O exchangeable, OH); 7.39–7.62 and 8.03 (m, d, 9 H, Ar–H+pyrazole–H). Anal. Calcd for $C_{21}H_{17}BrN_{10}O_2$ (521.35): C, 48.38; H, 3.29; N, 26.87, Found: C, 48.00; H, 3.00; N, 26.60.

3.15. Preparation and culture of Hep G2 2.2.15 cells

The required cell line was made by transfection of Hep G2-cells with a plasmid containing multiple tandem copies of HBV genome (subtype ayw).[33] The 2.2.15 cell line was maintained in RPMI-1640 (Glutamax) culture media containing 100 IU/mL nystatin+380 μg/mL G418 (geneticin). The transfected Hep G2-2.2.15 cell line was kept in a tissue culture flask at 37 °C+5% CO_2. Subcultures were set up after a week by aspiration of the media from culture flask and washing the cells twice by PBS. A 10% versene–trypsin was added and the cells were incubated for 1 min at 37 °C.

The drug Lamivudine, which is a potent selective inhibitor of HBV replication,[34] was used as a standard for the comparative studies.

3.15.1. PCR-ELISA. The PCR reaction mixture contained 14 μL of extracted supernatant, 4 mmol/L $MgCl_2$, 10 μmol/L DIG-11-dUTP, 190 μmol/L dTTP, 200 μmol/L dATP, dGTP, dCTP, 1.5 U Taq polymerase, 20 mmol/L HCl (pH 8.4), 50 mmol/L KCl, 1 μmol/L HCID-1 primer (5′GGA AAG AAG TCA GAA GGC A3′) and 1 μmol/L HCID-2 (5′TTG GGG GAG GAG ATT AGG TT3′), in a total volume 50 μL. PCR reaction conditions were 32 cycles of 1 min at 94 °C, 30 s at 58 °C and 30 s at 72 °C+3 s for each cycle in a thermal circler as described in literature.[35]

3.15.2. Cytotoxicity assay. A colorimetric assay for living cells should utilize a colorless substrate that is modified to a colored product by any living cells, but not by dead cells or tissue-culture medium. 3-(3,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) is the attractive candidate for this purpose. The cytotoxic effect of the compounds was accessed by culturing the Hep G2-2.2.15 cells in the presence of compounds, using a MTT-assay.[36]

3.15.3. Calculation of IC_{50}, CC_{50}, and SI. The 50% inhibitory concentration of antiviral drugs (IC_{50}) was determined by interpolation from the plots of amount of DNA copies versus antiviral drug concentrations. The 50% cytotoxic effect (CC_{50}) was calculated from the average viability of the cells with concentration of drugs. The selective index (SI) could be calculated as CC_{50}/IC_{50}.

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