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Isolation and identification of the intermediates during pyrazole formation of some carbohydrate hydrazone derivatives

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Abstract

Reaction of the oxidation product of L-ascorbic acid, dehydro-L-ascorbic acid, with *o*-phenylenediamine, followed by 2,4,6-trichlorophenylhydrazine (**3**) afforded 3-[1-(2,4,6-trichlorophenylhydrazono)-L-*threo*-2,3,4-trihydroxybut-1-yl]quinoxalin-2(1*H*)one (**4**), whose structure was deduced from studying its periodate oxidation, which gave the glyoxal derivative 3-[1-(2,4,6-trichlorophenylhydrazono)glyoxal-1-yl]quinoxalin-2(1*H*)one (**5**) that upon reduction afforded 3-[1-(2,4,6-trichlorophenylhydrazono)-2-hydroxyethy-1-yl]quinoxalin-2(1*H*)one (**6**). The reaction of **5** with **3** afforded the bishydrazone 3-[1,2-bis(2,4,6-trichlorophenylhydrazono)glyoxal-1-yl]quinoxalin-2(1*H*)one. The reaction of **5** with acetic anhydride in pyridine afforded the 2,3-dihydrofuro[2,3-*b*]quinoxaline derivative 2-acetoxy-3-[2-acetyl-2-(2,4,6-trichlorophenyl)hydrazono)]-2,3-dihydrofuro[2,3-*b*]quinoxaline. Acetylation of **4** with acetic anhydride in pyridine afforded the acyclic diacetate intermediate 3-[3,4-di-*O*-acetyl-2-deoxy-1-(2,4,6-trichlorophenylhydrazono)but-2-en-1-yl]quinoxalin-2(1*H*)one (**12**), which was also obtained from the reaction of **4** with boiling acetic anhydride. Compound **12** rearranged under the reaction conditions to give the pyrazole derivatives 3-[5-(acetoxymethyl)-1-(2,4,6-trichlorophenyl)pyrazol-3-yl]quinoxalin-2(1*H*)one (**14**) and 2-acetoxy-3-[5-(acetoxymethyl)-1-(2,4,6-trichlorophenyl)pyrazol-3-yl)]quinoxaline (**15**), as well as the 2,3-dihydrofuro[2,3-*b*]quinoxaline derivative 2-(2-acetoxyethen-2-yl)-3-[2-(2,4,6-trichlorophenyl)hydrazono]-2,3-dihydrofuro[2,3-*b*]quinoxaline. Acetylation of 3-[5-(hydroxymethyl)-1-(2,4,6-trichlorophenyl)pyrazol-3-yl]quinoxalin-2(1*H*)one (**16**) with acetic anhydride in pyridine or **12** with boiling acetic anhydride afforded **15** and **16**, respectively. Treatment of **4** with diluted sodium hydroxide afforded the pyrazolo[2,3-*b*]quinoxaline (flavazole) derivative 1-(2,4,6-trichlorophenyl)-3-(L-*threo*-glycerol-1-yl)pyrazolo[2,3-*b*]quinoxaline whose acetylation afforded the acetyl derivative 3-(2,3,4-tri-*O*-acetyl-L-*threo*-glycerol-1-yl)-1-(2,4,6-trichlorophenyl)pyrazolo[2,3-*b*]quinoxaline. The assigned structures were based on spectral analysis. The activity of compound **4** against hepatitis B virus has been studied.

Keywords: L-Ascorbic acid; (2,4,6-Trichlorophenylhydrazone); Quinoxalin-2(1*H*)one; 2,3-Dihydrofuro[2,3-*b*]quinoxaline; [But-2-en-1-yl]quinoxalin-2(1*H*)one; Pyrazole; Pyrazolo[2,3-*b*]quinoxaline

1. Introduction

Much attention has been devoted to the chemistry of the 2,3,4-tetrahydrofurantrione, obtained from the oxidation of L-ascorbic acid, as an excellent precursor for constructing heterocyclic rings either by retaining the carbon skeleton of the furanone ring or by rearrangement via its opening [1–10]. El Ashry et al. [6–9] have extensively studied the role of carbohydrates as organic raw materials for the synthesis of heterocycles. Although acetylation with boiling acetic anhydride of sugar

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hydrazones did not lead to pyrazole ring formation, but instead gave the respective peracetyl derivatives [11,12], the corresponding sugar osazones gave pyrazole derivatives under similar conditions [13–17]. On the other hand, the pyrazolyl quinoxaline derivative could be obtained under the same conditions from the product of the reaction of dehydro-L-ascorbic acid with *o*-phenylenediamine and arylhydrazines namely, 3-[1-(arylhydrazono)-L-*threo*-2,3,4-trihydroxybut-1-yl]quinoxalin-2(1*H*)one [6,7,18,19]. In this paper, the intermediates during the pyrazole formation from derivatives of the latter compound could be isolated, which sheds some light on the mode of the reaction.

2. Results and discussion

The reaction of L-*threo*-2,3-hexodiulosono-1,4-lactone, obtained from the oxidation of L-ascorbic acid (**1**) with *p*-benzoquinone, with 1 molar equivalent of *o*-phenylenediamine (**2**), followed by 2,4,6-trichlorophenylhydrazine (**3**), afforded a yellow product that showed in its IR spectrum an amide carbonyl absorption band at 1660 cm^{-1} and gave an elemental analysis that agreed with the molecular formula $C_{18}H_{15}Cl_3N_4O_4$. Consequently, the product was assigned the acyclic structure 3-[1-(2,4,6-trichlorophenylhydrazono)-L-*threo*-2,3,4-trihydroxybut-1-yl]quinoxalin-2(1*H*)one (**4**), rather than the hydrated form of the anhydro structure 2,2′-anhydro-[2-hydroxy-3-(1-(2,4,6-trichlorophenylhydrazono)-L-*threo*-2,3,4-trihydroxybutyl]quinoxaline] [2,19] (Scheme 1). Although its mass spectrum showed the highest ion peak at *m*/*z* 440 that agreed with the anhydro structure, this ion can be attributed to the loss of a molecule of water from the molecular ion peak of **4**. Thus, a confirmation for the acyclic nature of **4** becomes desirable. This was achieved from the results of its periodate oxidation that afforded a yellow crystalline product whose structure was established by spectral analysis (see Section 3) to be 3-[1-(2,4,6-trichlorophenylhydrazono)glyoxal-1-yl]quinoxalin-2(1*H*)one (**5**) favoring the acyclic nature of **4**.

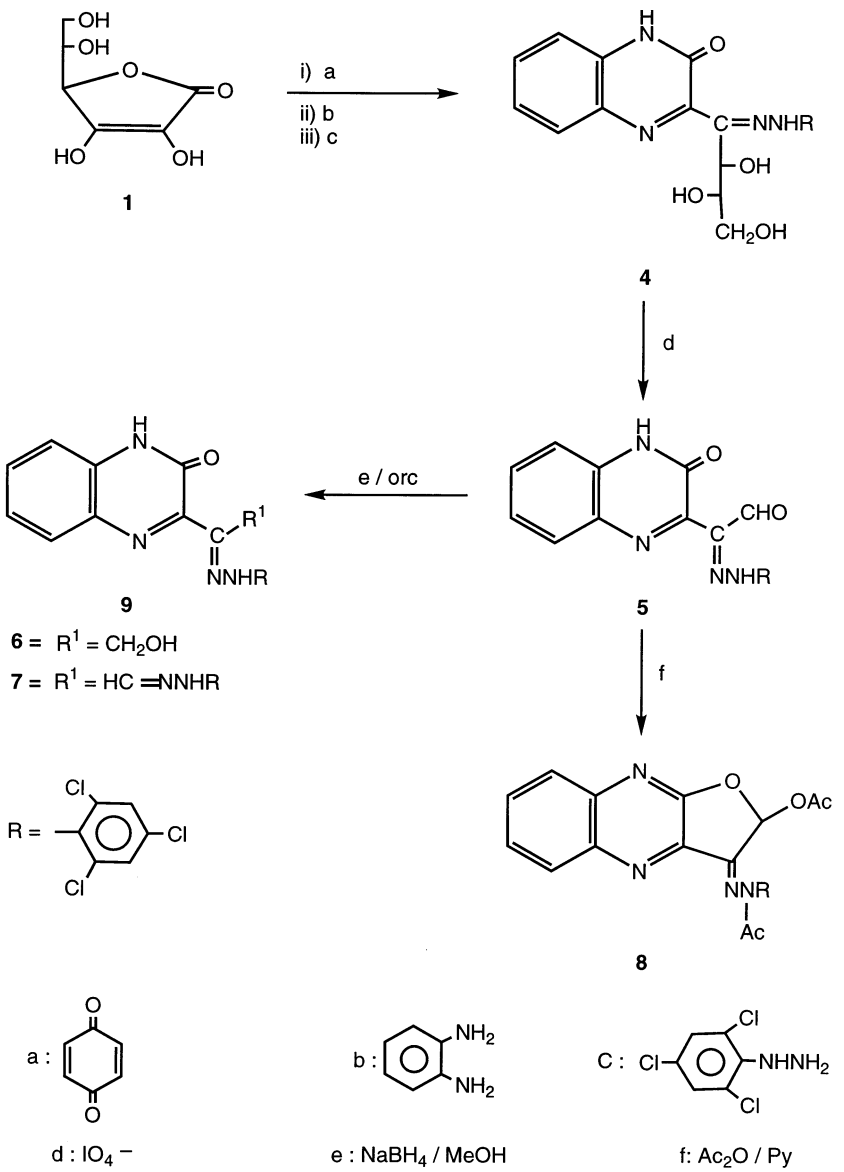
The reduction of **5** with sodium borohydride afforded an orange crystalline product 3-[1-(2,4,6-trichlorophenylhydrazono)-2-hydroxyeth-1-yl]quinoxalin-2(1*H*)one (**6**), and its reaction with **3** gave the bishydrazone derivative **7**. The FAB mass spectra of **6** and **7** showed molecular ion peaks at *m*/*z* 397 and 589, respectively, which were in agreement with their assigned structures. Inspection of the ^1H NMR spectral data of **6** and **7** measured in Me_2SO-d_6 revealed that both compounds may exist in their solutions in alternated chelated structures resulting from intramolecular hydrogen bonding (chelate N–H bond), as well as a solvent-bonded N–H [20–22].

The reaction of **5** with acetic anhydride in pyridine gave the 2,3-dihydrofuro[2,3-*b*]quinoxaline derivative **8** whose structure was deduced from its mode of formation as reported by El Ashry and co-workers [8,18,19] for the construction of the 2,3-dihydrofuro[2,3-*b*]-quinoxaline skeleton. The ^1H NMR spectrum of **8** showed two singlets due to the OAc and NAc and a singlet at δ 6.28, which was attributed to H-2 of the furan ring; such downfield shift was due to its attachment to a carbon bearing two heteroatoms. The formation of **8** could be explained by the possible interaction of the C-2 oxygen of the quinoxaline ring on the aldehydic carbonyl group with elimination of a water molecule and subsequent acetylation of the formed hydroxyl group, as well as the NH of the hydrazone moiety.

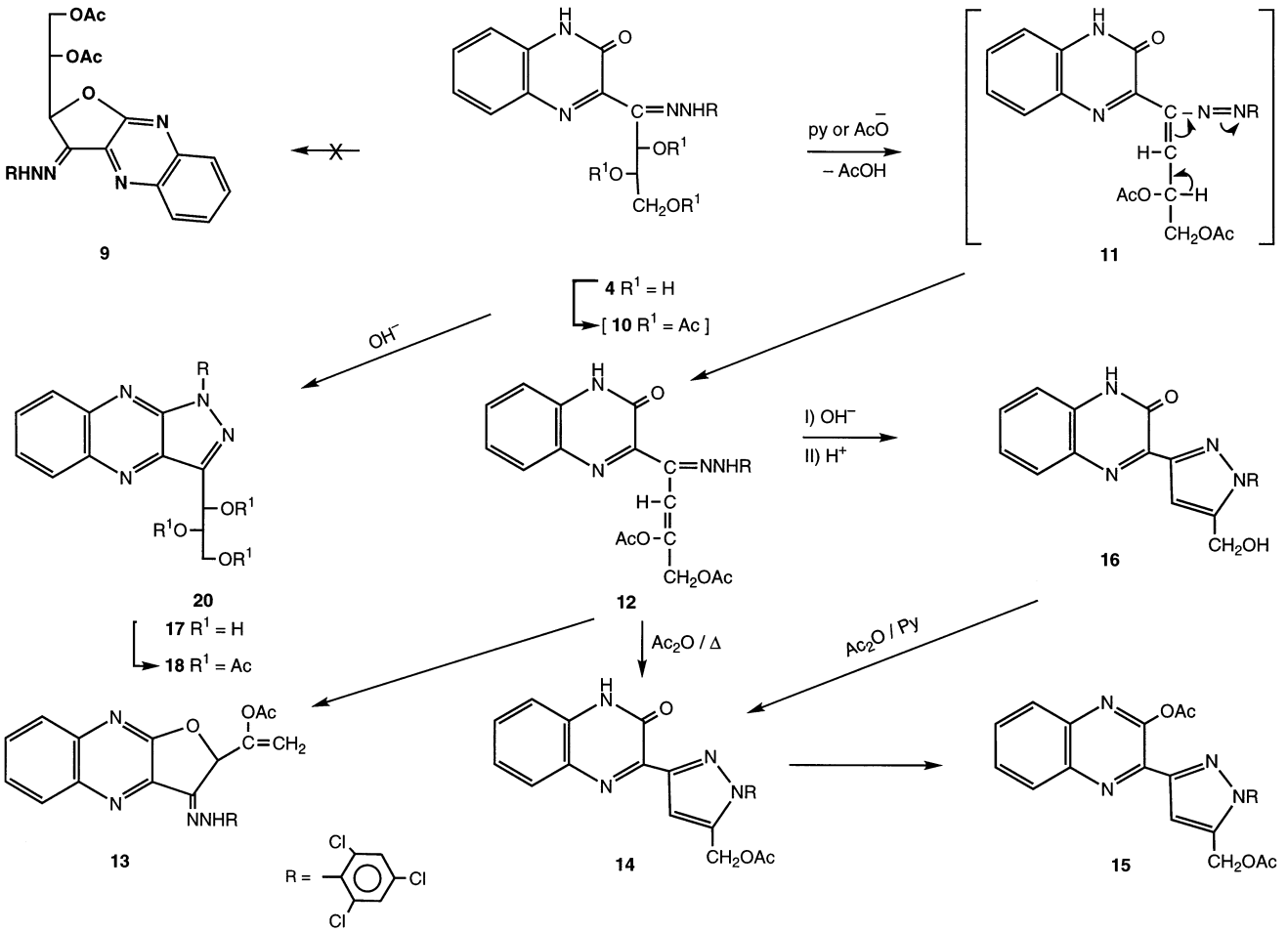
In order to gain definitive proof of the acyclic nature of **4**, as well as a better understanding of its mode of transformation to the pyrazole derivative [6,7], the acetylation of **4** under various conditions was studied. Thus the treatment of **4** with acetic anhydride in pyridine afforded an orange product **12**, which would be expected to be the triacetate **10** [19] or the diacetate **9** [2], but the spectral data ruled out this expectation. The IR spectrum of **12** showed acetoxy and amide carbonyl absorption bands at 1745 and 1661 cm^{-1}, respectively, indicating its acyclic nature, which was also established from its ^1H NMR spectrum. Two exchangeable protons were assigned at the downfield region at δ 11.70 and

13.95 and attributed to the NH protons of the amide and the hydrazone moieties, respectively. At the lower-frequency region only two acetyl signals were assigned at δ 2.01 and 2.20, and since the two singlets at δ 5.10 (2 H) and δ 7.07 (1 H) were attributed to methylene protons and a methine proton each without coupling, respectively, the structure **12** rather than the other possible structures was assigned for the product. The formation of the diacetate **12** can be explained taking into consideration the mechanism suggested for the cyclization of the sugar osazone acetate [16]. The first step involved the formation of the expected triacetate **10**, which undergoes a stepwise elimination of a molecule of acetic acid under the basic conditions of the reaction with the formation of the azoalkene intermediate **11** (Scheme 2). The formation and isolation of azoalkenes from sugar hydrazones is a well-known reaction [23]. However, in this case the product was tautomerized to the isolated hydrazono alkene namely, 3-[3,4-di-*O*-acetyl-2-deoxy-1-(2,4,6-trichlorophenylhydrazono)but-2-en-1-yl]quinoxalin-2(1*H*)one (**12**).

Acetylation of the analogues of **4** with boiling acetic anhydride gave pyrazolyl derivatives of the type **14** [6,7]. However, the reaction of **4** with acetic anhydride under the same conditions gave a mixture of products that could be



Scheme 1.



Scheme 2.

separated, and the products were identified as **12**, **13**, **14** and **15**. The structures of 3-[5-(acetoxymethyl)-1-(2,4,6-trichlorophenyl)pyrazol-3-yl]quinoxalin-2(1*H*)one (**14**) and 2-acetoxy-3-[5-(acetoxymethyl)-1-(2,4,6-trichlorophenyl) pyrazol-3-yl]quinoxaline (**15**) were assigned based on their spectral characteristics (see Section 3).

The structure of red product **13** was deduced from its FAB mass spectrum, which showed a molecular ion peak at m/z 464, agreeing with the elemental analysis data for the molecular formula $C_{20}H_{13}Cl_3N_4O_3$. Its IR spectrum showed the absence of the amide carbonyl absorption and the presence of OAc absorption at 1737 cm^{-1}. The ^1H NMR spectrum of **13** showed a singlet at δ 2.20 corresponding to an acetyl group. The two singlets at δ 4.85 (2 H) and 6.30 (1 H) were attributed to a methylene and a methine proton, respectively. The latter appeared at a similar location to that of the chemical shift of the C-2 proton of **8**. At the downfield region only one exchangeable proton was assigned to the singlet at δ 13.90 due to the hydrazono NH proton. Accordingly the structure **13** was assigned for the product and its formation can be explained by an intramolecular nucleophilic attack of the C-2-enolized oxygen of the amide group to the C-2 of the butenyl moiety of the intermediate diacetate **12** with rearrangement of the double bond and subsequent elimination of a molecule of acetic acid with ring closure to give the furoquinoxaline derivative **13**.

Ring closure of **12** to 3-[5-(hydroxymethyl)-1-(2,4,6-trichlorophenyl)pyrazol-3-yl]quinoxalin-2(1*H*)one (**16**) was induced under alkaline conditions. Thus, treatment of **12** with aqueous sodium hydroxide solution under reflux, followed by acidification with acetic acid, afforded **16** as a pale-yellow crystalline product.

On the other hand, treatment of **12** with boiling acetic anhydride afforded the expected pyrazolyl derivatives **14** and **15**. Acetylation of **16** with acetic anhydride in pyridine afforded a mixture of two products that were identical as **14** and **15**, as confirmed from their spectral data. Accordingly, from the above data the formation of the pyrazole derivatives **14** and **15** can now be explained to be formed from the intermediate diacetate **12** that undergoes an elimination of a molecule of acetic acid from the acetoxy group on C-3 and N–H proton of the hydrazone moiety with the formation of **14**, which was acetylated at the enolized amide group to give **15** under the reaction conditions.

Ring closure to pyrazolo[2,3-*b*]quinoxaline (flavazole) **17** has been attempted by treatment of **4** with dilute aqueous sodium hydroxide. The FAB mass spectrum of **17** showed a protonated molecular ion peak at *m*/*z* 441. Its IR spectrum showed the disappearance of the amide carbonyl absorption as well as the NH absorption bands, which is in agreement with its formation.

Acetylation of **17** with acetic anhydride in pyridine afforded a yellow hygroscopic acetyl derivative **18** whose structure was assigned based on its spectral data (see Section 3).

The favored conformation of acyclic sugar derivatives has been studied based on the vicinal proton–proton coupling constants [24–28]. Thus, examination of the extended zig-zag conformation of **18** indicated that H-1 and H-2 are in a gauche arrangement, which should require the $J_{1,2}$ value to be <4 Hz. However, $J_{1,2}$ was found to be 8.0 Hz, indicating that H-1 and H-2 are in an antiparallel disposition. This could be achieved by the rotation around the C-1–C-2 bond with a better steric arrangement for the bulky substituents at C-1. The $J_{2,3}$ of 4 Hz and $J_{2,3'}$ of 7 Hz indicated that both of H-3 and H-3′ would be in a gauche and antiparallel disposition to that of H-2.

Compound **4** was tested for its activity against hepatitis B virus (HBV) in Hep G_2 2.2.15 cells. The concentration of the tested compound was 10 μM. It showed high viral replication inhibition values of 89.5, 88.1 and 87.1% after 1, 2 and 3 weeks, respectively. It has a low cytotoxicity (7.7%).

3. Experimental

Melting points were determined with a Melt–Temp apparatus and are uncorrected. IR spectra were recorded for the compounds in a matrix of KBr with a Unicam SP1025 spectrophotometer. ^{1}H NMR spectra were determined with a Bruker AC 250 MHz spectrometer. The chemical shifts are expressed on the δ (ppm) scale using Me_4Si as the standard. Mass spectra were recorded using electron ionization (EI) on a Finnigan MAT 312 spectrometer and fast-atom bombardment (FAB) on a Kratos MS 50 spectrometer. Thin-layer chromatography (TLC) was performed on E. Merck Silica Gel 60 F_{254}. The spots were detected by their characteristic color and by UV light 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, Alexandria University.

*3-[1-(2,4,6-Trichlorophenylhydrazono)-*L*-threo-2,3,4-trihydroxybut-1-yl]quinoxalin-2-(1*H*)one* (**4**).—A mixture of L-ascorbic acid (**1**) (4.4 g, 25 mmol) and *p*-benzoquinone (2.7 g, 25 mmol) in EtOH (40 mL) was stirred for 90 min at room temperature (rt). The resulting solution was treated with a soln of *o*-phenylenediamine (**2**) (2.7 g, 25 mmol) in MeOH (25 mL) and water (100 mL), and heated until boiling. Then a solution of 2,4,6-trichlorophenylhydrazine (**3**) (5.28 g, 25 mmol) in EtOH (100 mL) was added. The reaction mixture was boiled for 5–10 min, whereupon a yellow crystalline product was separated out. It was filtered, washed with EtOH, dried, and recrystallized from EtOH to give a yellow crystals of **4** (8.4 g, 74% yield): mp 225–227 °C; IR (KBr): 3366 (OH), 3175, 3085 (NH), 1646 (OCN), 1540, 1514 cm^{-1} (C=N). EIMS: *m*/*z* (%): 442 (8, $[M^{+\bullet}+2-H_2O]$), 440 (8, $[M^{+\bullet}-H_2O]$), 426 (7, $[M^{+\bullet}+4-2\ H_2O]$), 424 (25, $[M^{+\bullet}+2-2\ H_2O]$), 422 (26, $[M^{+\bullet}-2\ H_2O]$), 396 (21, 426 − CH_2-O), 394 (36, 424 − CH_2O), 392 (47, 422 − CH_2-O), 381 (21), 379 (32), 377 (37), 366 (7, 426 − CH_2OH–CHO), 364 (24, 424 − CH_2OH–CHO), 362 (28, 422 − CH_2OH–CHO), 199 (26, $[C_6H_3Cl_3N]^+$), 197 (91, $[C_6H_3$-

$Cl_3N]^+$), 195 (100, $[C_6H_3Cl_3N]^+$). Anal. Calcd for $C_{18}H_{15}Cl_3N_4O_4$ (457.5): C, 47.23; H, 3.30; N, 12.24. Found: C, 47.42; H, 2.89; N, 12.24.

3-[1-(2,4,6-Trichlorophenylhydrazono)glyoxal-1-yl]quinoxalin-2(1H)one (**5**).—A mixture of **4** (2.20 g, 4.80 mmol) and sodium metaperiodate (3.2 g, 15 mmol) in distilled water (100 mL) was stirred overnight at rt. The product obtained was filtered off, washed with water, dried, and crystallized from EtOH to give yellow crystals of **5** (1.5 g, 79% yield): mp 258–260 °C; IR (KBr) 3170, 3112 (NH), 1710 (CO), 1665, (CON) 1609 and 1534 cm^{-1} (C=N); FABMS ($CHCl_3$–NBOH): *m/z* (%) 399 (9, $[MH]^+ + 2$), 397 (9%, $[MH]^+$); ^1H NMR (Me_2SO-d_6): δ 7.25–7.40, 7.50–7.85 (2 m, 6 H, Ar–H), 9.5 (s, 1 H, HC=O), 11.20 (s, 1 H, D_2O exchangeable, CONH), 12.6 (s, 1 H, D_2O exchangeable, =N–NH). Anal. Calcd for $C_{16}H_9Cl_3N_4O_2 \cdot H_2O$ (413.64): C, 46.45; H, 2.68; N, 13.54. Found: C, 46.05; H, 2.70; N, 13.23.

3-[1-(2,4,6-Trichlorophenylhydrazono)-2-hydroxyeth-1-yl]quinoxalin-2(1H)one (**6**).—To a stirred solution of **5** (0.3 g, 0.63 mmol) in abs EtOH (50 mL), $NaBH_4$ (0.18 g, 4.7 mmol) was added. The reaction mixture was stirred for 2 h at rt. It was then acidified with AcOH and diluted with water. The product separated out and was filtered, washed with water, and crystallized from EtOH to give an orange crystalline product **6** (0.2 g, 80% yield): mp 232–234 °C; IR (KBr) 3416 (OH), 3113 NH, 1667 (CON), 1549 cm^{-1} (C=N); FABMS ($CHCl_3$–NBOH) *m/z* (%), 399 (14, $[M + 2]^+$) 397 (18, $[M]^+$), 381 (13, $[M + 2 - H_2O]^+$), 379 (14, $[M - H_2O]^+$); ^1H NMR (Me_2SO-d_6): δ 4.60, 4.73 (2 d, 2 H, CH_2), 6.05, 6.40 (2 m, 1 H, D_2O exchangeable, OH) 7.27–7.70, 7.52–7.80, 7.85 (2 m, d, 6 H, Ar–H), 10.24, 12.45, 12.84 (3 s, 1 H, D_2O exchangeable NH) and 11.24 (s, 1 H, D_2O exchangeable OCNH). Anal. Calcd for $C_{16}H_{11}Cl_3N_4O_2$ (397.65): C, 48.32; H, 2.78; N, 14.09. Found: C, 47.83; H, 2.99; N, 13.84.

3-[1,2-Bis(2,4,6-trichlorophenylhydrazono)-glyoxal-1-yl]quinoxalin-2(1H)one (**7**).—A solution of **5** (0.1 g, 0.25 mmol) in EtOH (40 mL) was boiled under reflux with 2,4,6-trichlorophenylhydrazine (**3**) (0.053 g, 0.25 mmol) and two drops of AcOH for 30 min. The resulting solution was filtered and left to cool, whereupon a red crystalline product separated out. It was filtered, washed with EtOH, and recrystallized from EtOH to give reddish crystals of **7** (0.1 g, 67% yield): mp 215–217 °C; IR (KBr) 3170 (NH) and 1648 cm^{-1} (CON); FABMS: ($CHCl_3$–NBOH) *m/z* (%); 593 (13, $[M + 4]^+$) 591 (24, $[M + 2]^+$); 589 (24, $[M]^+$); ^1H NMR (Me_2SO-d_6): δ 7.27–7.40, 7.53–7.85 (2 m, 8 H, Ar–H), 8.35 (s, 1 H, HC=N), 10.27, 10.84, 12.50, 12.70 and 12.90 (5 s, 2 H, D_2O exchangeable 2 NH), 11.25 (s, 1 H, D_2O exchangeable, OCNH). Anal. Calcd for $C_{22}H_{12}Cl_6N_6O$ (589.08): C, 44.85; H, 2.05; N, 14.26. Found: C, 45.36; H, 1.78; N, 13.73.

2-Acetoxy-3-[2-acetyl-2-(2,4,6-trichlorophenyl)hydrazono)]-2,3-dihydrofuro[2,3-b]-quinoxaline (**8**).—A solution of **5** (0.33 g, 0.83 mmol) in pyridine (5 mL) was cooled in an ice-bath, then treated with Ac_2O (5 mL) with stirring. The reaction mixture was left at rt overnight, then poured onto crushed ice. The product separated out, was filtered off, washed repeatedly with water, and dried. It was crystallized from EtOH in yellow crystals (0.27 g, 82% yield): mp 191–192 °C; IR (KBr) 1772 (OAc), 1721 (NAc), 1637 and 1618 cm^{-1} (C=N); ^1H NMR (Me_2SO-d_6): δ 2.05 (s, 3 H, $OCOCH_3$), 2.70 (s, 3 H, $NCOCH_3$), 6.28 (s, 1 H, H-2), 7.70–7.90, 8.05, 8.15, 8.20 (m, 3 d, 6 H, Ar–H). Anal. Calcd for $C_{20}H_{13}Cl_3N_4O_4 \cdot H_2O$ (497.72): C, 48.26; H, 3.03; N, 11.26. Found: C, 48.73; H, 2.89; N, 10.68.

3-[3,4-Di-O-acetyl-2-deoxy-1-(2,4,6-trichlorophenylhydrazono)but-2-en-1-yl]quinoxalin-2(1H)one (**12**).—A solution of **4** (0.3 g, 0.65 mmol) in pyridine (7 mL) was treated with Ac_2O (7 mL) at 0 °C. The reaction proceeded as above to give an orange crystalline product **12** (0.3 g, 88% yield): mp 175–178 °C; IR (KBr) 3150 (NH) 1745 (OAc), 1661 (OCN) and 1610 cm^{-1} (C=N); ^1H NMR ($CDCl_3$): δ 2.01 (s, 3 H, $COCH_3$), 2.20 (s, 3 H, $COCH_3$), 5.10 (s, 2 H, CH_2), 7.07 (s, 1 H, CH), 7.35–7.45, 7.53–7.60 and 7.85 (2 m, s, 6 H, Ar–H), 11.70 (bs, 1 H, D_2O exchangeable, OCNH) and 13.95 (s, 1 H, D_2O exchangeable =NNH). Anal. Calcd for $C_{22}H_{17}Cl_3N_4O_5$ (523.76): C, 50.44; H, 3.27; N, 10.69. Found: C, 50.61; H, 3.30; N, 11.27.

Reaction of **4** *with boiling acetic anhydride*. A suspension of compound **4** (0.5 g, 1.09 mmol) in Ac_2O (25 mL) was heated under reflux. The reflux was continued for 30 min after the solid was dissolved. The resulting solution was cooled and then poured into ice-cold water. The product obtained was filtered off, washed with water, and dried. The crude product was chromatographed on silica gel by eluting with 2:1 EtOAc–petroleum ether to give **12**, **13**, **14** and **15**.

3-[3,4-Di-O-acetyl-2-deoxy-1-(2,4,6-trichlorophenylhydrazono)but-2-en-1-yl]quinoxalin-2-(1H)one (**12**).—The compound was identical to that obtained from **4** and Ac_2O–pyridine (0.1 g, 18% yield).

3-[5-(Acetoxymethyl-1-(2,4,6-trichlorophenyl)pyrazol-3-yl]quinoxalin-2 (1H)one (**14**).—The compound was obtained as pale-yellow needles (0.05 g, 10% yield): mp 195–197 °C; IR (KBr) 1744 (OAc), 1656 (OCN), 1610 and 1578 cm^{-1} (C=N); ^1H NMR ($CDCl_3$): δ 2.05 (s, 3 H, $COCH_3$), 5.05 (s, 2 H, CH_2), 7.30–7.43, 7.55, 7.80, 8.10 (m, d, s, d, 7 H, Ar–H + CH) and 11.65 (s, 1 H, D_2O exchangeable, OCNH). Anal. Calcd for $C_{20}H_{13}Cl_3N_4O_3$ (463.70): C, 51.80; H, 2.82; N, 12.08. Found: C, 52.13; H, 2.88; N, 11.99.

2-Acetoxy-3[5-(acetoxymethyl)-1-(2,4,6-trichlorophenyl)pyrazol-3-yl)]quinoxaline (**15**).—The compound was separated as a white crystalline product (0.13 g, 24% yield): mp 155–156 °C; IR (KBr) 1756 (OAc) and 1612, 1570 cm^{-1} (C=N); ^1H NMR ($CDCl_3$): δ 2.00 (s, 3 H, $COCH_3$), 2.30 (s, 3 H, $COCH_3$), 5.0 (s, 2 H, CH_2) and 7.40–7.55, 7.73–7.80, 7.95–8.02, 8.17–8.20 (4 m, 7 H, Ar–H + CH). Anal. Calcd for $C_{22}H_{15}Cl_3N_4O_4$ (505.74): C, 52.24; H, 2.99; N, 11.07. Found: C, 52.37; H, 3.06; N, 10.63.

2-(2-Acetoxyethen-2-yl)-3-[2-(2,4,6-trichlorophenyl)hydrazono]-2,3-dihydrofuro[2,3-b]quinoxaline (**13**).—The compound was separated as a red crystalline product (0.05 g, 10% yield): mp 205–207 °C; IR (KBr): 3077 (NH), 1737 (OAc) and 1576 cm^{-1} (C=N) FABMS ($CHCl_3$–NBOH): m/z (%) 464 (20, $[M]^+$), 403 (6, $[M-OAc]^+$); ^1H NMR ($CDCl_3$): δ 2.20 (s, 1 H, $COCH_3$), 4.85 (s, 2 H, CH_2), 6.30 (s, 1 H, CH), 7.35, 7.67–7.78, 7.85, 7.95 (s, m, 2 d, 6 H, Ar–H) and 13.90 (s, 1 H, D_2O exchangeable, NH). Anal. Calcd for $C_{20}H_{13}Cl_3N_4O_4$ (463.70): C, 51.80; H, 2.83; N, 12.08. Found C, 51.71; H, 2.89; N, 11.90.

3-[5-(Hydroxymethyl)-1-(2,4,6-trichlorophenyl)pyrazol-3-yl]quinoxalin-2-(1H)one (**16**).—A solution of **12** (1 g, 1.9 mmol) in EtOH (25 mL) was treated with a NaOH solution (1 N, 25 mL). The reaction mixture was heated under reflux for 4 h. The clear solution obtained was neutralized with AcOH and left to cool, whereupon a yellow product separated out. The product was filtered, washed with water, and crystallized from EtOH to give **16** (0.46 g, 58% yield): mp 235–237 °C; IR (KBr) 3440 (OH), 1662 (OCN), 1553 cm^{-1} (C=N); ^1H NMR ($CDCl_3$): δ 4.55 (s, 2 H, CH_2), 7.20–7.45, 7.7 and 8.05 (m, s and d, 7 H, Ar–H + C–H), 11.05 (s, 1 H, D_2O exchangeable, NH). Anal. Calcd for $C_{18}H_{11}Cl_3N_4O_2$ (421.67): C, 51.27; H, 2.63; N, 13.28. Found: C, 51.09; H, 3.08; N, 12.86.

3-[5-(Acetoxymethyl)-1-(2,4,6-trichlorophenyl)pyrazol-3-yl]quinoxalin-2 (1H)one (**14**) *and 2-acetoxy-3-[5-acetoxymethyl)-1-(2,4,6-trichlorophenyl) pyrazol-3-yl]quinoxaline* (**15**)

Method A. A suspension of **12** (0.1 g, 0.19 mmol) in Ac_2O (10 mL) was heated under reflux for 20 min. The clear solution obtained was poured onto crushed ice with stirring, the product obtained was filtered off, washed with water, and dried. The crude product was fractionally crystallized from cold acetone to give **14** (0.02 g, 23% yield) and **15** (0.04 g, 42% yield).

Method B. A cold solution of **16** (0.12 g, 0.28 mmol) in pyridine (3 mL) was treated with Ac_2O (5 mL). The reaction mixture was left at rt overnight, then poured onto crushed ice and proceeded as in Method A to give **14** (0.03 g, 23% yield) and **15** (0.06 g, 43% yield).

*1-(2,4,6-Trichlorophenyl)-3-(*L-threo-*glycerol-1-yl)pyrazolo[2,3-*b*]quinoxaline* (**17**).—A suspension of **4** (0.5 g, 1.09 mmol) in NaOH solution (0.01 M, 500 mL) was heated under reflux for 3 h. The resulting solution was filtered off while hot, then left to cool, whereupon a yellow crystalline product was obtained. The product was filtered, washed with water, dried and crystallized from EtOH (0.2

g, 63% yield): mp 125–127 °C; IR (KBr) 3406 (OH), 1551 cm^{-1} (C=N); FABMS ($CHCl_3$–NBOH) *m/z* (%); 443 (90, $[MH+2]^+$), 441 (90, $[MH]^+$), 423 (3, $[MH-H_2O]^+$), 391 (5, $324-CH_2OH$). Anal. Calcd for $C_{18}H_{13}Cl_3N_4O_3$ (439.68): C, 49.16; H, 2.98; N, 12.74. Found: C, 48.83; H, 3.36; N, 12.27.

3-(2,3,4-Tri-O-*acetyl*-L-threo-*glycerol-1-yl)-1-(2,4,6-trichlorophenyl)pyrazolo[2,3-*b*]quinoxaline* (**18**).—A cold solution of **17** (0.2 g, 0.45 mmol) in pyridine (5 mL) was treated with Ac_2O (5 mL). The reaction was then carried out as for **8**. The crude product was chromatographed on silica gel. Elution with 1:1 EtOAc–petroleum ether gave **18** as a yellow crystals (0.27 g, 65% yield): mp 58–60 °C; IR (KBr) 1752 cm^{-1} OAc. ^1H NMR ($CDCl_3$): δ 2.00, 2.07, 2.22 (3 s, 9 H, 3 $COCH_3$), 4.51 (dd, 1 H, $J_{2,3'}$ 7 Hz, H-3′), 4.20 (dd, 1 H, $J_{2,3}$ 4 Hz, $J_{3,3'}$ 13 Hz, H-3), 5.96–6.04 (m, 1 H, H-2), 6.82 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 7.55–7.60, 7.75–7.90, 8.10, 8.31 (2 m, 2 d, 6 H, Ar–H), Anal. Calcd for $C_{24}H_{19}Cl_3N_4O_6$ (565.79): C, 50.94; H, 3.38; N, 9.90. Found: C, 50.37; H, 3.72; N, 9.52.

Biological activity studies.—Maintenance media (RPMI/Glutamax, 93%; penicillin + streptomycin, 1%; gentamycin, 1%, fetal calf serum, 5% and geneticin 4 mL/100 mL media) were added to the cell culture (Hep G_2 2.2.15) together with the tested compound (concentration 10 μM). The supernatant media were collected after 1, 2 and 3 weeks for the tested compound and the controls (Hep G_2 2.2.15 cells without added compound). The DNA replication was estimated by the PCR (polymerase chain reaction) technique, which was carried out in three steps: extraction of DNA from supernatant, amplification of DNA by using thermal cycler, and finally detection by the DIG-ELISA technique. The percentage inhibition was calculated by the relation % inhibition = [(inhibition of compound/inhibition of control) − 1] × 100.

The percentage cytotoxicity could be estimated by the relation between the number of living and dead cells counted after 3 weeks by hemocytometer. The viral replication inhibition values were 89.5, 88.1 and 87.1% after 1, 2 and 3 weeks; the cytotoxicity was 7.7%.

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