Synthesis and biological activity of some new fused pyrazole-c-nucleosides

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Received 15 December 2017
Accepted 18 February 2018

Egyptian Pharmaceutical Journal 2018, 17:104–108

Background and objectives
Owing to the high antiviral, antibiotic, antitumor, and antifungal activities associated with various C-nucleosides, much attention has been paid on the synthesis of this class of compounds. We synthesized new pyrazole-C-nucleosides through one-pot reaction of open sugar, phenyl hydrazine hydrochloride, and different bases in presence of acetic acid and sodium acetate as a catalyst. These nucleosides, containing pyrazole moiety, have been marked as biologically active compounds.

Keywords:
chemotherapeutic effects, one-pot synthesis, pyrazole-C-nucleosides

Egypt Pharmaceut J 17:104–108

1687-4315

Introduction

Among the wide variety of pyrazole derivatives that have been explored for developing potential pharmacologically active compounds, pyrazoles fused with different heterocycles, that are known to contribute to various chemotherapeutic effects have emerged as antimicrobial [1,2], antifungal [3], and antiviral agents [4]. In addition, some fused pyrazole derivatives were reported to induce various antileukemic [5], antitumor [6,7] and antiproliferative [8,9] activities. Moreover, fused pyrazole-C-nucleosides have strong biological activities against many diseases. So we prepared new nucleosides through one-pot synthesis of three compounds (one sugar and two bases).

Materials and methods

Chemistry

All melting points were measured on an electrothermal melting point apparatus and are uncorrected. The infrared spectra were recorded in potassium bromide pellets on a Pye Unicam (Cambridge, England) SP 3–300 or Shimadzu (Shimadzu, Tokyo, Japan) FT-IR 8101 PC infrared spectrophotometer. The ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) spectra were recorded in DMSO-d6 on a GEMINI-200 spectrometer using TMS as the internal reference. Results are expressed in ppm and coupling constants (*J*) in Hertz. Mass spectra were measured on a GCMS-QP 1000 EX spectrometer at 70 eV. Elemental analyses were carried out at the Micro analytical Center of Cairo University, Giza, Egypt.

General procedure for the preparation of compounds (2a–d)

To a solution of sugar, containing d(+) mannose, d (+) glucose, d(+) arabinose, and d(+) xylose (0.01 mol), in water (100 ml), the base 5-amino uracil and 5-amino 6-methyl uracil (0.01 mol), phenyl hydrazine hydrochloride (7.2 g; 0.05 mol), acetic acid (6 ml, 60%), and 0.5-g sodium acetate were added. The reaction mixture was heated at 60°C on a water bath for 2–3 h. The reaction mixture was cooled and yellow precipitate was filtered off, and washed successively with ethyl acetate. The compounds gave one spot on thin layer chromatography (TLC).

3-(1,2,3-Trihydroxypropyl)-1-phenyl-4,8-dihydropyrazolo [3,4-*b*]pyrimido[5,4-*e*][1,4]oxazin-7(^{1}*H*)-one (2a)

2a was recrystallized from methanol-ethylacetate as a yellow needle, m. p. 180–183°C, with molecular formula $C_{16}H_{14}N_5O_5$ (mol. wt. 356.13 mg). ^{1}H-NMR (DMSO) δ: 3.42 (m, ^{2}H, H-3′), 3.48 (m, ^{1}H, H-2′), 3.88 (d, ^{1}H, H-1′), 4.35 (m, ^{1}H, OH), 4.89 (m, ^{1}H, OH), 5.22 (t, ^{1}H, OH), 6.22 (dd, ^{1}H, CH), 6.81–7.26 (m, ^{5}H, Ar-H), 10.1 (s, ^{1}H, NH); yield 80%, m. p. 58–59°C; IR: 3190–3300 (3OH), 3150 (NH), 1724 (C=O), and 1605 (C=N).

3-(1,2,3-Trihydroxypropyl)-1-phenyl,5-methyl-4,8-dihydropyrazolo[3,4-*b*]pyrimido[5,4-e][1,4]oxazin-7(¹*H*)-one (2b)

2b was recrystallized from methanol-ethylacetate as a yellow needle, m. p. 193–196°C, with molecular formula $C_{17}H_{16}N_5O_5$ (mol. wt. 370.16 mg), ^{1}H-NMR (DMSO) δ: 3.31 (m, ^{2}H, H-3′), 3.42 (m, ^{1}H, H-2′), 3.7 (d, ^{1}H, H-1′), 4.25 (m, ^{1}H, OH), 4.32 (m, ^{1}H, OH), 5.11 (t, ^{1}H, OH), 6.10 (dd, ^{1}H, CH), 6.51–7.81 (m, ^{5}H, Ar-H), 11.2 (s, ^{1}H, NH); yield 83%, m. p. 71–72°C; IR: 3200–3310 (3OH) 3143 (NH), 1712 (C=O), and 1612 (C=N).

3-(1,2,3-Trihydroxypropyl)-1-phenyl-4,8-dihydropyrazolo [3,4-*b*]pyrimido[5,4-e][1,4]oxazin-7(¹*H*)-one (2c)

2c was recrystallized from ethanol as a white needle, m. p. 190–192°C, with molecular formula $C_{15}H_{12}N_5O_4$ (mol. wt. 326.14). ^{1}H-NMR (DMSO) δ: 3.47 (m, ^{1}H, H-2′), 3.56 (d, ^{1}H, H-1′), 4.33 (m, ^{1}H, OH), 4.46 (m, ^{1}H, OH), 6.71–7.26 (m, ^{9}H, Ar-H), 10.1 (s, ^{1}H, NH). ^{13}C-NMR (DMSO) δ: 73 (C-2′), 79 (C-1′), 114, 120, 129 (Ar-C), 145 (CN), 150 (CO) and 211 (C=O).

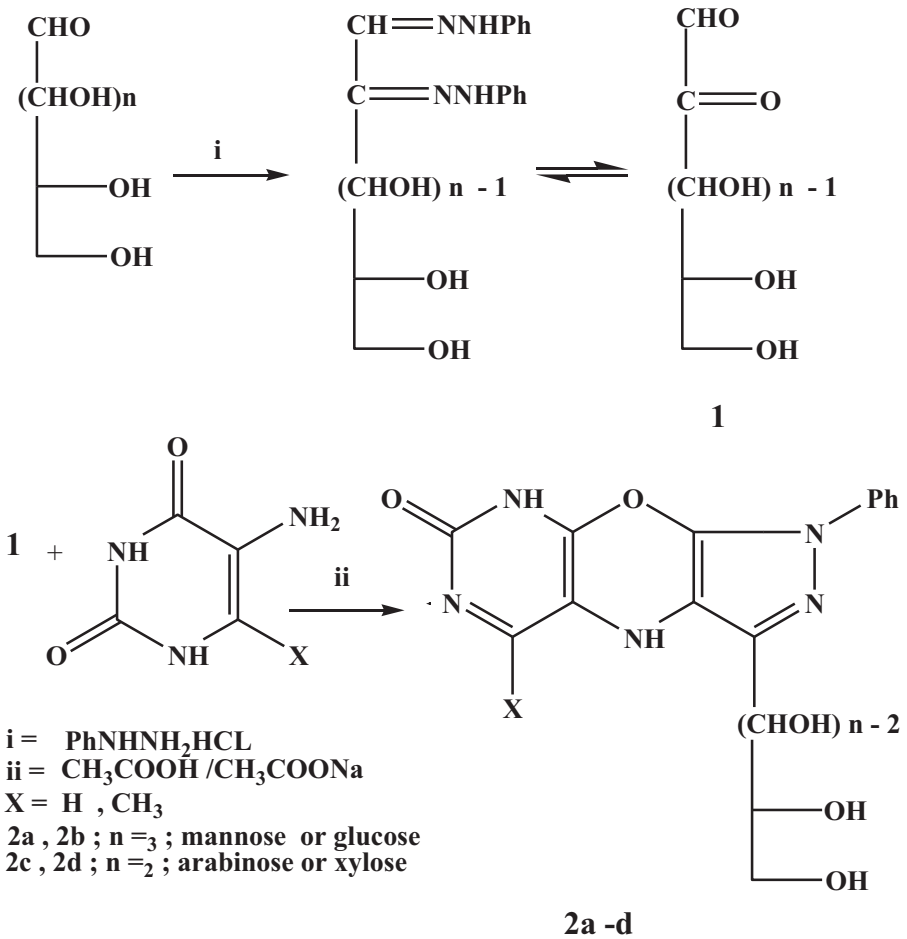
3-(1,2-Dihydroxyethyl)-1-phenyl, 3-methyl-4,8-dihydropyrazolo[3,4-*b*]pyrimido[5,4-e][1,4]oxazin-7(¹*H*)-one (2d)

2d was recrystallized from ethanol as a white needle, m. p. 188–189°C, with molecular formula $C_{16}H_{14}N_5O_4$ (mol. wt. 340.13). ^{1}H-NMR (DMSO) δ: 3.49 (m, ^{1}H, H-2′), 3.63 (d, ^{1}H, H-1′), 4.12 (m, ^{1}H, OH), 4.42 (m, ^{1}H, OH), 6.63–7.22 (m, ^{9}H, Ar-H), 10.65 (s, ^{1}H, NH). ^{13}C-NMR (DMSO) δ: 50(CH3), 76 (C-2′), 78 (C-1′), 115, 119, 127 (Ar-C), 144 (CN), 151 (CO), and 212 (C=O).

General procedure for the preparation of compounds (3a–d)

To a solution of the sugar d(+) mannose, glucose, arabinose, and xylose (0.01 mol) in water (100 ml), the base (2-amino imidazole) (0.01 mol), phenyl hydrazine hydrochloride (7.2 g; 0.05 mol), acetic acid (6 ml, 60%), and 0.5 g sodium acetate were added. The reaction mixture was heated at 60°C on a water bath for 2–3 h. The reaction mixture was cooled and yellow precipitate was filtered off, and washed successively with ethyl acetate. The compounds gave one spot on TLC.

Scheme 1



Synthesis of compounds **2a-d**. sc1

3-(1,2,3-Trihydroxypropyl)-1-phenyl-pyrazolo-[2,3-b]) benzimidazole) (3a)

3a was recrystallized from methanol as a yellow needle, m. p. 185–187°C, with molecular formula $C_{19}H_{17}N_5O_3$ (mol. wt. 363.17). ^{1}H-NMR (DMSO) δ: 3.25 (m, ^{2}H, H-3′), 3.47 (m, ^{1}H, H-2′), 3.70 (d, ^{1}H, H-1′), 4.33 (m, ^{1}H, OH), 4.53 (m, ^{1}H, OH), 4.76 (t, ^{1}H, OH), 6.81–7.36 (m, ^{9}H, Ar-H), 10.1 (s, ^{1}H, NH), EI, MS: m/z (%)=(363 M^+, 75); yield 80%, m. p. 58–59°C; IR: 318–3310 (3OH), 3140 (NH), and 1602 (C=N).

3-(1,2,3-Trihydroxypropyl)-3-methyl,1-phenyl-pyrazolo-[2,3-b])benzimidazole) (3b)

3b was recrystallized from methanol as a yellow needle, m. p.178–180°C, with molecular formula $C_{20}H_{19}N_5O_3$ (mol. wt. 377.16). ^{1}H-NMR (DMSO) δ: 3.25 (m, ^{2}H, H-3′), 3.47 (m, ^{1}H, H-2′), 3.70 (d, ^{1}H, H-1′), 4.33 (m, ^{1}H, OH), 4.53 (m, ^{1}H, OH), 4.76 (t, ^{1}H, OH), 6.81–7.36 (m, ^{9}H, Ar-H), 10.1 (s, ^{1}H, NH)., EI, MS: m/z (%)=(363 M^+, 75); yield 80%; m. p. 58–59°C; IR: 3200–3320 (3OH), 3150 (NH), and 1605 (C=N).

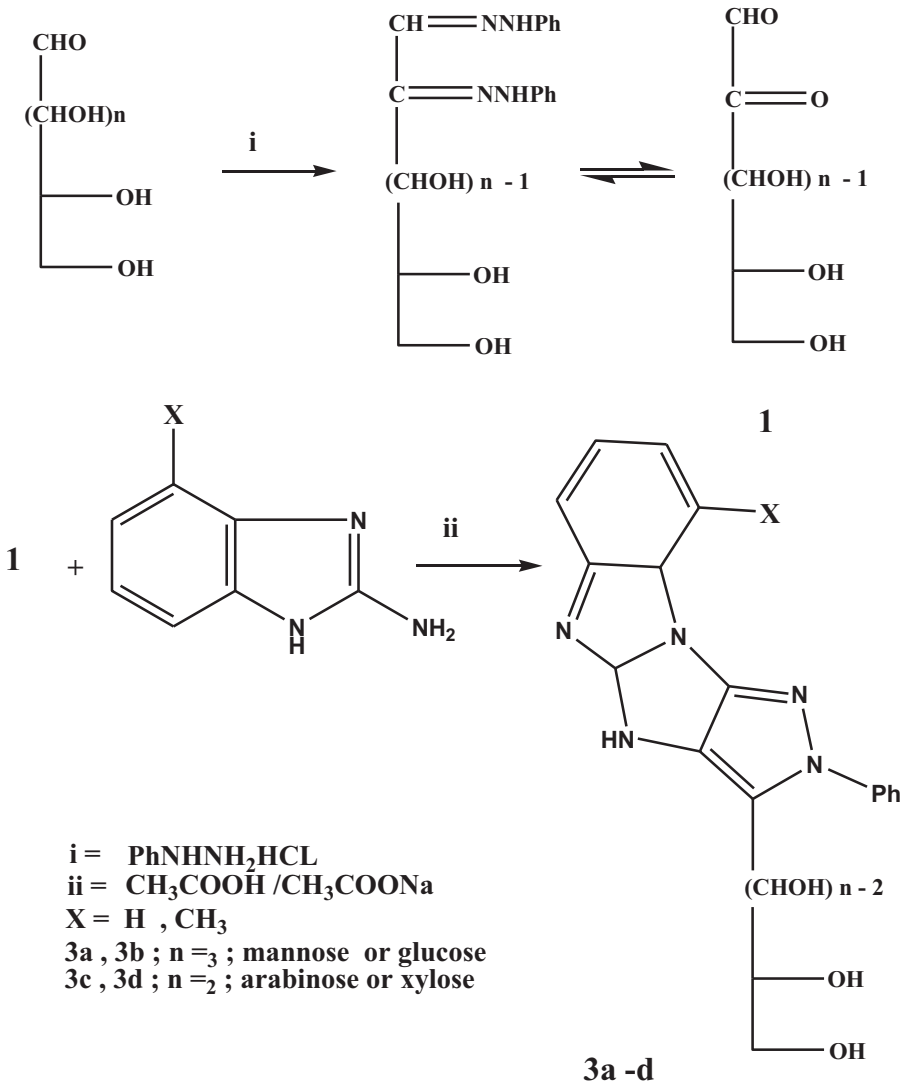
3-(1,2-Dihydroxyethyl)-1-phenyl-pyrazolo-[2,3-b]) benzimidazole) (3c)

3c was recrystallized from methanol as a yellow needle, m. p. 175–176°C, with molecular formula $C_{18}H_{15}N_5O_2$ (mol. wt. 333.11). ^{1}H-NMR (DMSO) δ: 3.25 (m, ^{2}H, H-3′), 3.47 (m, ^{1}H, H-2′), 3.70 (d, ^{1}H, H-1′), 4.33 (m, ^{1}H, OH), 4.53 (m, ^{1}H, OH), 4.76 (t, ^{1}H, OH), 6.81–7.36 (m, ^{9}H, Ar-H), 10.1 (s, ^{1}H, NH), EI, MS: m/z (%)=(363 M^+, 75); yield 80%; m. p. 58–59°C; IR: 3235–3300 (2OH), 3221 (NH), and 1611 (C=N).

3-(1,2-Dihydroxyethyl)-3-methyl,1-phenyl-pyrazolo-[3,4-b])benzimidazole (3d)

3d was recrystallized from methanol as a yellow needle, m. p. 173–175°C., with molecular formula $C_{19}H_{17}N_5O_2$

Scheme 2



Synthesis of compounds **3a-d**. sc2

Table 1 Calculation of 50% inhibitory concentration of antiviral drugs, 50% cytotoxic effect, and selective index

Compound nos	HBV DNA (IC_{50}) (μmol/l)	Hep G2 2.2.15 (CC_{50}) (μmol/l)	SI
2a	25.41	>100	3.25
2b	36.32	>100	3.36
2c	34.32	>100	3.56
2d	22.32	>100	3.78
3a	26.74	>100	3.95
3b	26.33	>100	3.90
3c	16.51	>100	7.82
3d	24.74	>100	7.49

CC_{50}, 50% cytotoxic effect; HBV, hepatitis B virus; IC_{50}, 50% inhibitory concentration of antiviral drugs; SI, selective index.

(mol. wt. 347.14). ^{1}H-NMR (DMSO) δ: 3.37 (m, ^{1}H, H-2′), 3.70 (d, ^{1}H, H-1′) 4.33 (m, ^{1}H, OH), 4.76 (t, ^{1}H, OH), 6.81–7.36 (m, ^{9}H, Ar-H), 10.1 (s, ^{1}H, NH). ^{13}C-NMR (DMSO) δ: 73 (C-2′), 79 (C-1′), 111, 114, 119, 128, 150 (Ar-C); yield 80%; m. p. 58–59°C; IR: 3190–3286 (2OH), 3166 (NH), and 1603 (C=N).

Biological activity

The potential target for antiviral chemotherapy is the reverse transcription step in hepatitis B virus (HBV) life cycle [10–14]. The minus strand of HBV is synthesized by reverse transcription of pregenome using the endogenous viral reverse transcriptase. It is shown that reverse transcription enzyme leads to incorporate nucleotide analogues more efficient than cellular DNA polymerase [15–17]. These nucleotide analogues are competitive inhibitors of the reverse transcriptase with the nucleosides pool in the cells cytoplasm in minus strand synthesis.

The recent development of nucleoside analogues has represented a breakthrough in the research for selective antiviral activities. Among these agents, for example, Lamivudine acts as a retroviral inhibitor [18]. It has activity against HBV replication both *in vitro* and *in vivo*.

Calculation of 50% inhibitory concentration of antiviral drugs, 50% cytotoxic effect, and selective index

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

Results and discussion

In this work, 5-amino, 6-methyl uracil reacted with sugars and phenyl hydrazine hydrochloride to give fused pyrazole-C-nucleosides. Thus, 5-amino uracil reacts with d(+) mannose, d(+) glucose, d(+) arbinose, d(+) xylose, and phenyl hydrazine hydrochloride to give **2a–d**. It should be noted that the products form with multicomponent reactions in one step via Michael addition (Scheme 1).

Moreover, 2-amino, 4-methyl imidazole reacted with d (+) mannose, d(+) glucose, d(+) arbinose, d(+) xylose, and phenyl hydrazine hydrochloride in the presence of aqueous acetic acid to give **3a–d** (Scheme 2).

Conclusion

The result of the antiviral screening against HBV of selected compounds indicated that compounds **2a**, **2b**, **2c**, **2d**, **3a**, **3b**, **4c**, and **3d** showed low inhibition and high cytotoxicity with selective indices 3.25–7.49 (Table 1): CC_{50}, IC_{50}, and selective index of compounds **2a**, **2b**, **2c**, **2d**, **3a**, **3b**, **4c**, and **3d**.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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