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The Reaction of Digitoxin and Digoxin with Cyanoacetic Acid Hydrazide: Synthesis of Coumarin, Thiazole, Thiophene and Pyridine Derivatives with Potential Biological Activities

Senot H. Doss[1], **Wagnat W. Wardakhan**[2], **and Nadia A. Louca**[1]

[1]*Hormones Department, National Research Center, Dokki, Giza, Egypt and* [2]*National Organization for Drug Control & Research, P.O. 29 Cairo, Egypt*

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The reaction of either Digitoxin or Digoxin with cyanoacetic acid hydrazide gave the hydrazone derivatives **3a** and **3b** respectively. The reactivity of the latter products towards chemical reagents was studied to give heterocyclic derivatives with potential biological activities.

Key words: Digitoxin, Digoxin, Cyanoacetic acid hydrazide

INTRODUCTION

Digitoxin as a cardiac glycoside binds strongly to the steroid recognition site on the cardiac glycoside acceptor (Templeton *et al.*, 1992, 1993, 1991). The most potent pregnane derivatives identified so far are C-3 glycosides that are cardio tonic and kindly not shared by the digitalis drugs (Templeton *et al.*, 1988; Smyth *et al.* 1992). They may also be involved in the regulation of vascular tone (Woolfson *et al.* 1992). In this work we plan to show the reactivity of Digitoxin and Digoxin towards chemical reagents to synthesis heterocyclic and fused heterocyclic derivatives with expected biological and pharmaceutical uses (Kantoci *et al.*, 1997; Adumczyk *et al.*, 1996; Templeton *et al.*, 1993; Gobbini *et al.*, 1997). Due to the foresaid biological activities, which attracts our attention towards the uses of the title reagent in heterocyclic synthesis with the aim of production of new derivatives, which showed better effects in human body with the decrease of the harmful effects.

MATERIALS AND METHODS

All melting points were uncorected. IR spectra (KBr disks) were recorded on a pye unicam sp-100 spectrometer. H'NMR spectra (DMSO: *dc, as* solvent) were obtained on a varian A.90 spectrometer using SiMe4 internal standard. Chemical shifts were expressed as (6 ppm) value. Analytical data were obtained from the microanalytical data unit at Cairo University, and National Research Center. All described compounds showed the characteristic protons signals ^1H NMR of either digitoxin or digoxin for which the characteristic signals due to the heterocyclic ring formation or the new chain is described below.

22-[cyanoacetic acid hydrazono] digitoxin (3a) and 22-[cyanoacetic acid hydrazono] digoxin (3b) (General procedure)

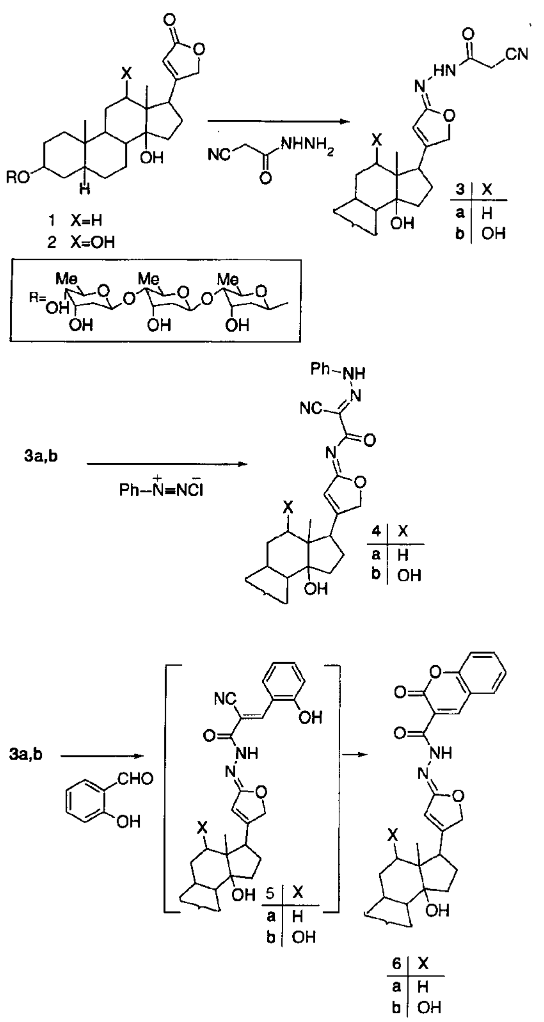
Method A

To a solution of either **1** (0.76 g, 0.001 mol) or **2** (0.78 g, 0.001 mol) in methanol (20 ml) cyanoacetic acid hydrazide (0.09 g, 0.001 mol) was added [prepared by the addition of hydrazine hydrate (5 ml, 0.1 mol) to ethyl cyanoacetate (11.3 g, 0.1 mol) with stirring at room temperature]. Hydrochloric acid (0.5 ml) was added to mixture and the reaction mixture was stirred at 25°C for 30 min then the solid product formed was collected by filtration.

Compound **3a**: White crystals from dilute methanol, yield 83%, m.p. 173°C. IR: 3400-3200 (OH , NH), 2980, 2890 (CH_3, CH_2) , 2225 (C=N), 1700 (C=O), 1645-1630 cm^{-1} (C=N, C=C). ^1H NMR (δ ppm): 4.98 (s, 2H, CH;), 7.76 (s, 1H, NH). $C_{44}H_{67}O_{13}N_3$: Calcd: C, 62.48 H, 7.92 N, 4.87. Found: C, 62.37 H, 7.81 N, 4.79.

Compound **3b**: White crystals from ethanol, yield 75%,

Correspondence to: Dr. Senot H. Doss, Hormones Department, National Research Center, Dokki, Giza, Egypt
E-mail: rmmohareb@hotmail.com



Scheme 1. Synthesis of compound **3**, **4**, and **6**

m.p. 195-198°C. IR: 3350-3340 (OH, NH), 2980, 2890 (CH_3, CH_2), 2220 (CN), 1680 (C=O), 1650-1630 cm^{-1} (C=N, C=C). ^{1}H NMR (δ ppm): 4.50 (s, 2H, CH_2), 6.01 (s, 1H, NH). $C_{44}H_{67}O_{14}N_3$: Calcd: C, 61.32 H, 7.78 N, 4.9. Found: C, 61.31 H, 7.65 N, 4.70.

Method B

To a solution of either **12a** (1 g, 0.001 mol) or **12b** (1.0 g, 0.001 mol) in ethanol (20 ml) containing piperidine (0.5 ml) ethyl cyanoacetate (0.1 g, 0.001 mol) was added. The reaction mixture was heated under reflux for 3 h then left to cool. The solid product formed upon dilution with water was collected by filtration.

22-[α-phenylhydrazonocyanoacetic acid hydrazono] digitoxin (4a) and 22-[α-phenylhydrazonocyanoacetic acid hydrazono] digoxin (4b)

To a cold solution of either **3a** (0.84 g , 0.001 mol) or **3b** (0.86 g , 0.001 mol) at 0°C in ethanol (20 ml) containing sodium acetate (0.08 g, 0.001 mol), a solution of benzenediazonium chloride [prepared by the addition of sodium nitrite solution (0.06 g, 0.001 mol) to a cold suspension of aniline (0.08 ml, 0.001 mol) containing the appropriate amount of hydrochloric acid with stirring] was added with stirring. The reaction mixture was poured into ice / water and the formed solid product was collected by filtration.

Compound **4a**: Brown crystals from ethanol, yield 77 %, m.p. 98°C. IR: 3350-3340 (NH , OH), 2980, 2890 (CH_3, CH,), 2225 (CN), 1700 cm^{-1} (C=O). ^{1}H NMR (δ ppm): 6.5, 7.0 (2s , 2H, 2NH), 7.50-7.89 (m, 5H, C_6H_5) $C_{50}H_{71}O_{13}N_5$: Calcd: C, 63.22 H, 7.48 N, 7.3. Found: C, 63.12 H, 7.31 N, 7.2.

Compound **4b**: Brown crystals from ethanol, yield 77 %, m.p. 218°C. IR: 3440-3200 (OH, NH), 2980, 2890 (CH_3, CH_2), 2220 (CN), 1695 (C=O), 1660-1645 cm^{-1} (C =N, C=C). ^{1}H NMR (δ ppm): 6.60, 6.85 (2s, 2H, 2NH), 7.35-7.70 (m, 5H, C_6H_5). $C_{50}H_{71}O_{14}N_5$: Calcd: C, 62.17 H.7.35 N, 7.25. Found: C, 62.05 H, 7.29 N, 7.19.

22-[coumarin-3-carbohydrazido] digitoxin (6a) and 22-[coumarin-3-carbohydrazido] digoxin (6b)

To a solution of either **3a** (0.84 g, 0.001 mol) or **3b** (0.86 g. 0.001 mol) in dimethyl formamide (20 ml) containing piperidine (0.5 ml), salicylaidehyde (0.1 g, 0.001 mol) was added. The reaction mixture was heated under reflux for 4h then left to cool, the solid product formed upon dilution with water was collected by filtration.

Compound **6a**: Yellow crystals from methanol, yield 80%, m.p. 185-188°C. IR: 3340-3200 (OH , NH), 2980 , 2890 (CH_3, CH_2), 1680 (C=O), 1650-1630 cm^{-1} (C=N, C=C). ^{1}H NMR (δ ppm): 6.99 (s, 1H, coumarin, H-4), 7.32-7.35 (m, 4H, C_6H_4), 8.75 (s, 1H, NH). $C_{51}H_{70}O_{15}N_2$: Calcd: C, 64.42 H.7.36. Found: C, 64.21 H, 7.1.

Compound **6b**: Yellow crystals from methanol, yield 80%, m.p. 175, 178 °C. IR: 3430-3300 (OH, NH), 2980, 2890 (CH_3, CH_2), 1720 (C=O), 1650-1645 cm^{-1} (C=N, C=C). ^{1}H NMR (δ ppm): 6.90 (s, 1H, coumarin H-4), 7.32-7.35 (m, 4H, C_6H_4), 8.75 (s, 1H, NH). $C_{51}H_{70}O_{16}N_2$: Calcd: C, 63.35 H, 7.24 N, 2.89. Found: C,63.15 H, 7.0 N, 2.71.

22-[3,5-diamino-2-cyano-4-carbohydrazidothiepheno] digitoxin (7a) and 22-[3,5-diamino-2-cyano-4-carbohydrazidothiopheno] digoxin (7b)

To a solution of either **3a** (0.84 g, 0.001 mol) or **3b** (0.86 g, 001 mol) or in ethanol (20 ml) containing triethylamine (0.5 ml), elemental sulfur (0.03 g, 0.001 mol) was added followed by malononitrile (0.06 g, 0.001 mol). The reaction mixture was heated under reflux for 3 h then poured into ice/water containing a few drops of hydrochloric acid and the formed solid product was collected by filtration.

Compound **7a**: Yellow crystals from methanol, yield 80

%, m.p. 142-144°C. IR: 3480-3320 (OH, NH_2, NH), 2980, 2885 (CH_3, CH_2), 2220 (CN), 1730 (C=O), 1645-1630 cm^{-1} (C=N, C=C). ^1H NMR (δ ppm): 4.40-4.50 (m, 4 H, $2NH_2$), 8.65 (s, 1H, NH). $C_{47}H_{69}O_{14}N_5S$: Calcd: C,59.85 H, 7.31 N, 7.42 S, 3.39. Found: C, 59.71 H, 7.30 N, 7.30 S, 3.25.

Compound **7b**: Brown crystals from ethanol, yield 77%, m.p. 188-190°C. IR: 3480-3320 (OH, NH, NH_2), 2980, 2885 (CH_3, CH_2), 2220 (C=N), 1730 (C=O), 1645-1630 cm^{-1} (C= N, C=C). ^1H NMR (δ ppm): 4.77, 5.70 (2s, 4H, 2 NH_2), 8.46 (s, 1 H, NH). $C_{47}H_{69}O_{14}N_5S$: Calcd: C, 58.81 H, 7.19 N, 7.29 S, 3.33 Found: C, 58.72 H, 7.1 N, 7.15 S, 3.19.

22-[1-phenyl-5-amino-2-thieno-4-carbohydrazidothiazolo] digitoxin (8a) and 22-[1-phenyl-5-amino-2-thieno-4-carbohydrazidothia-zolo]digoxin (8b)

To a solution of either **3a** (0.84 g, 0.001 mol) or **3b** (0.86 g, 0.001 mol) in ethanol (20 ml) containing triethylamine (0.5 ml), elemental sulfur (0.03 g, 0.001 mol) was added followed by phenyl isothiocyanate (0.1 g, 0.001 mol). The reaction mixture was heated under reflux for 4h then poured into ice/water containing a few drops of hydrochloric acid and the formed solid product was collected by filtration.

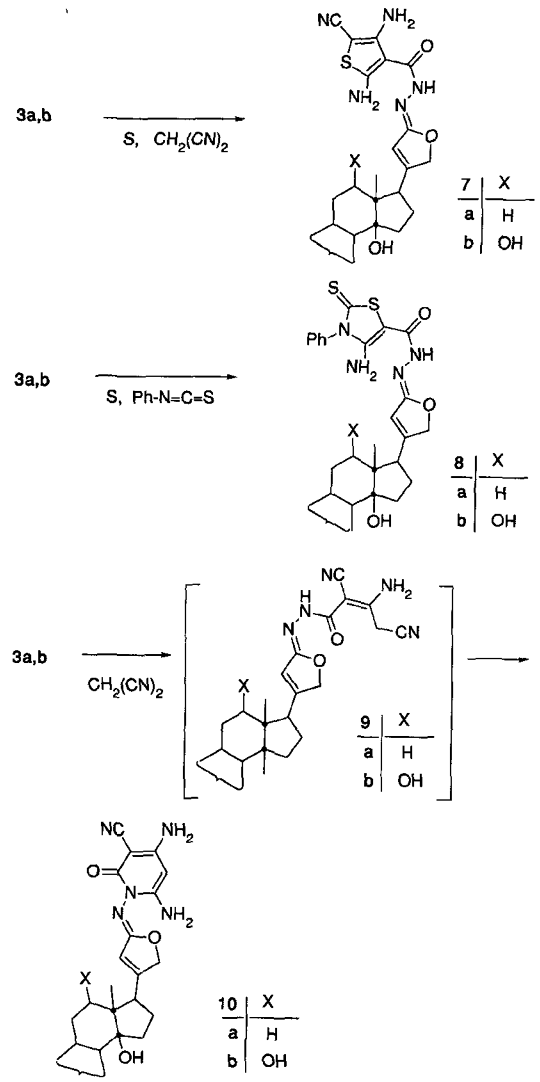
Compound **8a**: Yellow crystals from ethanol, yield 77%, m.p. 50°C. IR: 3400-3200 (OH , NH , NH_2), 3000 (aromatic CH), 2980, 2890 (CH_3, CH_2), 1730 (C=O), 1645-1630 cm^{-1} (C=N, C=C). ^1H NMR (δ ppm): 4.48 (s, 2H, NH_2), 7.56 (m, 2H, CH_2), 9.75 (s, 1H, NH). $C_{51}H_{72}O_{13}N_4S_2$: Calcd: C, 60.47 H, 7.13 N, 5.53 S, 6.43 Found: C, 60.31 H, 7.01 N, 5.41 S, 6.12.

Compound **8b**: White crystals from ethanol, yield 80%, m.p. 85-88°C. IR: 2980, 2890 (CH_3, CH_2), 1730 (C=0), 1650-1630 cm^{-1} (C=N, C=C). ^1H NMR (δ ppm): 4.48 (s, 2H, NH_2), 7.56 (m, 5H, C_6H_5), 9.75 (s, 1H, NH). $C_{51}H_{72}O_{14}N_4S_2$: Calcd: C, 59.53 H, 7.03 N, 5.44 S, 6.22 Found: C, 59.31 H, 6.80 N, 5.25 S, 6.05.

22-[*N*-imino-2,4-dimino-5-cyano-6-oxopyridino] digitoxin (10a) and 22-[*N*-imino-2,4-dimino-5-cyano-6-oxopyridino] digo-xin (10b)

To a solution of either **3a** (0.84 g, 0.001 mol) or **3b** (0.86 g, 0.001 mol), in ethanol (20 ml) malononitrile (0.6 g, 0.001 mol) was added, and the reaction mixture was heated under reflux for 3 h then poured into ice/water containing a few drops hydrochloric acid. The formed solid product was collected by filtration.

Compound **10a**: Yelow crystals from ethanol, yield 88%, m.p. 192-194°C. IR: 3400-3200 (OH, NH, NH,), 2225 (C=N), 2980, 2890 (CH_3, CH_2), 1750 (C=O), 1645-1630 cm^{-1} (C=N, C=C). ^1H NMR (δ ppm): 4.89, 4.59 (2s, 4H, $2NH_2$), 5.75 (s, 1H, pyridine H-3) $C_{47}H_{69}O_{13}N_5$: Calcd: C, 61.90 H, 7.57 N, 7.68. Found: C, 61.70 H, 7.45 N, 7.60.



Scheme 2. Synthesis of compound 7, 8, and 10

Compound **10b**: Yellow crystals from ethanol, yield 78%, m.p. 128-130°C. IR: 3480-3300 (OH, NH), 2980, 2890 (CH3, CH_2), 2225 (CN), 1750 (C=O), 1645-1630 cm^{-1} (C=N, C=C). ^1H NMR (δ ppm): 4.89, 4.54 (2s, 4H, $2NH_2$), 5.75 (s, 1H, pyridine H-5). $C_{47}H_{69}O_{14}N_5$: Calcd: C, 60.84 H, 7.44 N, 7.55. Found: C, 60.95 H, 7.39 N, 7.41.

22-[2-cyano-5-amino-3-ethoxycarbonyl-4-carbohydrazido] digitoxin (11)

To a solution of **3a** (0.76 g, 0.001 mol) in ethanol (20 ml) containing triethylamine (0.5 ml), elemental sulfur (0.03 g, 0.001 mol) was added followed by ethyl cyanoacetate (0.1 g, 0.001 mol). The reaction mixture was heated under reflux for 3 h then poured into ice/water containing a few drops of hydrochloric acid and the formed solid product was collected by filtration.

Compound **11**: Brown crystals from ethanol, yield 77%, m.p. 187-190°C. IR: 3340-3350 (OH, NH, NH;), 2980, 2890 (CH3, CH2), 2220 (C sN), 1750 (C=O), 1650-1635 cm^{-1} (C=N, C=C). $C_{50}H_{72}O_{15}N_4S$: Calcd: C, 60.07 H, 7.22 N, 5.60 S, 3.21. Found: C, 60.02 H, 7.11 N, 5.54 S, 3.08.

22-hydrazonodigitoxin (12a) and 22-hydrazonodigoxin (12b)

To a solution of either **1** (0.76 g, 0.001 mol) or **2** (0.78 g, 0.001 mol) in ethanol (20 ml), hydrazine hydrate (0.05 g, 0.001 mol) was added. The reaction mixture was heated under reflux for 6h then poured into ice/water. The solid product formed was collected by filtration.

Compound **12a**: White crystals from ethanol, yield 78%, m.p. 196°C. IR: 3400-3200 (OH, NH, NH_2), 2980, 2890 (CH_3, CH_2), 1650-1630 cm^{-1} (C=N,C=C). ^1H NMR (δ ppm): 4.67 (s, 2H, NH_2). $C_{41}H_{66}O_{12}N_2$: Calcd: C, 63.23 H, 8.48 N, 4.94. Found: C, 63.21 H, 8.45 N, 4.70.

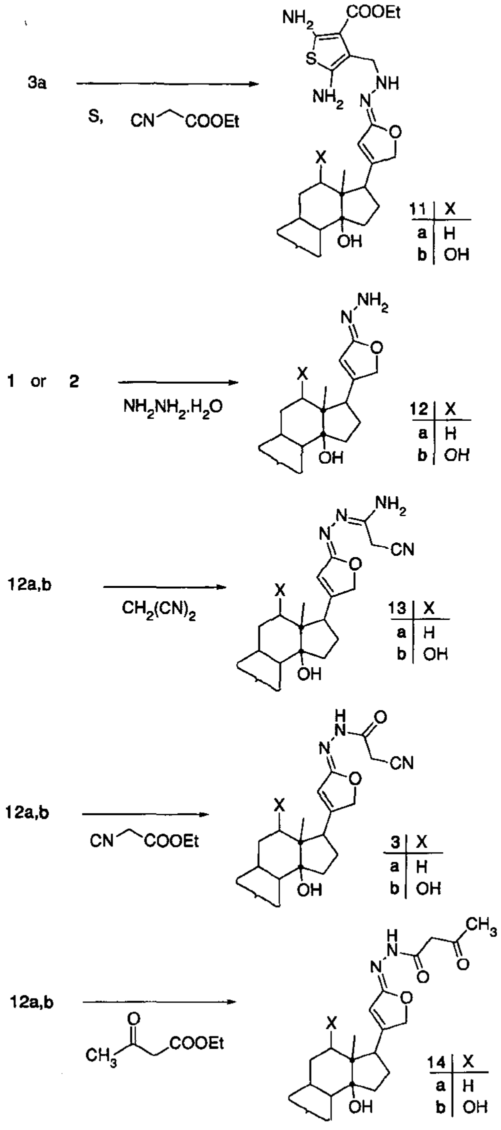
Compound **12b**: White crystals from ethanol, yield 78%, m.p. 198-200°C. IR: 3580-3200 (OH, NH, NH_2), 2980, 2890 (CH_3, CH_2), 1650-1630 cm^{-1} (C=N, C=C). ^1H NMR (δ ppm); 4.67 (s, 2H, NH_2). $C_{41}H_{66}O_{13}N_2$: Calcd.: C, 61.96 H, 8.33 N, 3.52. Found: C, 61.75 H, 8.12 N, 3.29.

22-[*p*-amino-*p*-ylidino-*p*ropienonitrilehydrazono] digitoxin (13a) and 22-[*p*-amino-*p*-ylidino-propienonitrilohydrazono] digoxin (13b)

To a solution of either **12a** (1.0 g, 0.001 mol) or **12b** (0.79 g, 0.001 mol) in ethanol (20 ml) containing piperidine (0.5 ml), malononitrile (0.6 g, 0.001 mol) was added. The reaction mixture was heated under reflux for 5h then poured into ice/water containing few drops of hydrochloric acid and the formed solid product was collected by filtration.

Compound **13a**: White crystals from methanol, yield 85%, m.p. 155-158°C. IR: 3400-3200 (OH, NH_2), 2980, 2890 (CH_3, CH_2), 2225 (CN), 1645-1630 cm^{-1} (C=N, C=C). ^1H NMR (δ ppm): 3.50 (s, 2H, CH_2), 3.60 (s, 2H, NH_2). $C_{44}H_{68}O_{12}N_4$: Calcd: C, 62.55 H, 8.05 N, 6.63. Found: C, 62.35 H, 8.01 N, 6.60.

Compound **13b**: Yellow crystals from dioxan, yield 82 %, m.p. 128-130°C. IR: 3480-3260 (OH, NH,), 2220 (CN), 1660-1635 cm^{-1} (C=N, C=C). ^1H NMR (δ ppm): 3.50 (s, 2H, CH_2), 3.60 (s, 2H, NH_2). $C_{44}H_{68}O_{13}N_4$: Calcd: C, 61.39 H, 7.9 N, 6.51. Found: C, 61.15 H, 7.7 N, 6.34.



Scheme 3. Synthesis of compound **11**, **12**, **13**, and **14**

22-[α-acetylaceticacid hydrazono] digitoxin (14a), 22-[α-acetyl acetic acid hydrazono] digoxin (14b), 22-[*N*-imino-4-methyl-2-amino-3-cyano-6-oxopyridino] digitoxin (16a), and 22-[*N*-imino-4-methyl-2-amino-3-cyano-6-oxo-pyridino] digoxin (16b)

To a solution of either **12a** (0.78 g, 0.001 mol) or **12b** (0.79 g, 0.001 mol) in methanol (20 ml) containing piperidine (0.5 ml), either ethyl acetoacetate (0.1 g, 0.001 mol) or malononitrile (0.66 g,. 01 mol) was added. The reaction mixture, in each case, was heated under reflux for 4 h then poured into ice/water containing few drops of hydrochloric acid and the formed solid product was collected by filtration. Compound **14a**: White crystals from ethanol, yield 80 %, m.p. 188-189°C. IR: 3400-3200 (OH, NH), 2980 , 2890 (CH_3, CH_2), 1750 (C=O), 1650 1630 cm^{-1} (C=N,C=C). ^1H NMR (δ ppm): 2.53 (s, 3H, CH_3), 5.80 (s, 2H, NH_2), 8.50 (s, 1H, NH). $C_{45}H_{70}O_{14}N_2$: Calcd: C, 62.64. H, 8.12 N, 3.24. Found: C, 62.51 H, 8.01 N, 3.15.

Compound **14b**: White crystals from ethanol, yield

78%, m.p. 165-167°C. IR: 3480-3250 (OH, NH), 2980, 2890 (CH_3, CH_2), 1720, 1700 (2C=O), 1660-1635 cm^{-1} (C=N, C=C). ^1H NMR (δ ppm): 2.53 (s, 3H, CH_3), 5.80 (s, 2H, CH_2), 8.50 (s, 1H, NH). $C_{45}H_{70}O_{15}N_2$: Calcd: C, 61.53 H, 7.97 N, 3.18. Found: C, 61.31 H, 7.82 N, 3.01.

Compound **16a**: Yellow crystals from dilute ethanol, yield 81 % m.p. 209-210°C. IR: 3400-3200 (OH, NH), 2980, 2890 (CH_3, CH_2), 2200 (CN), 1750 (C=O), 1630-1620 cm^{-1} (C=N, C=C). ^1H NMR (δ ppm): 2.52 (s, 3H, CH_3), 3.86 (s, 2H, NH_2), 7.50 (s, lH, pyridine H-5). $C_{48}H_{70}O_{13}N_4$: Calcd: C, 63.29 H, 7.69 N, 6.15. Found: C, 63.25 H, 7.58 N, 6.02.

Compound **16b**: Yellow crystals from ethanol, yield 80 %, m.p. 148-150°C. IR: 3480-3320 (OH, NH), 2980, 2890 (CH_3, CH_2), 2220 (CN), 1730 (C=O), 1655-1635 cm^{-1} (C=N, C=C). ^1H NMR (δ ppm): 2.52 (s, 3H, CH_3), 9.86 (s, 2H, NH_2), 7.50 (s, 1H, pyridine H-5) $C_{48}H_{70}O_{14}N_4$: Calcd.: C, 62.24 H, 7.52 N, 6.04. Found: C, 62.03 H, 7.36 N, 5.91.

22-[α-benzal-α-acetyl cyanoacetic acid hydrazono] digitoxin (17a) and 22-[α-benzal-α-acetylcyanoacetic acid hydrazono] digoxin (17b)

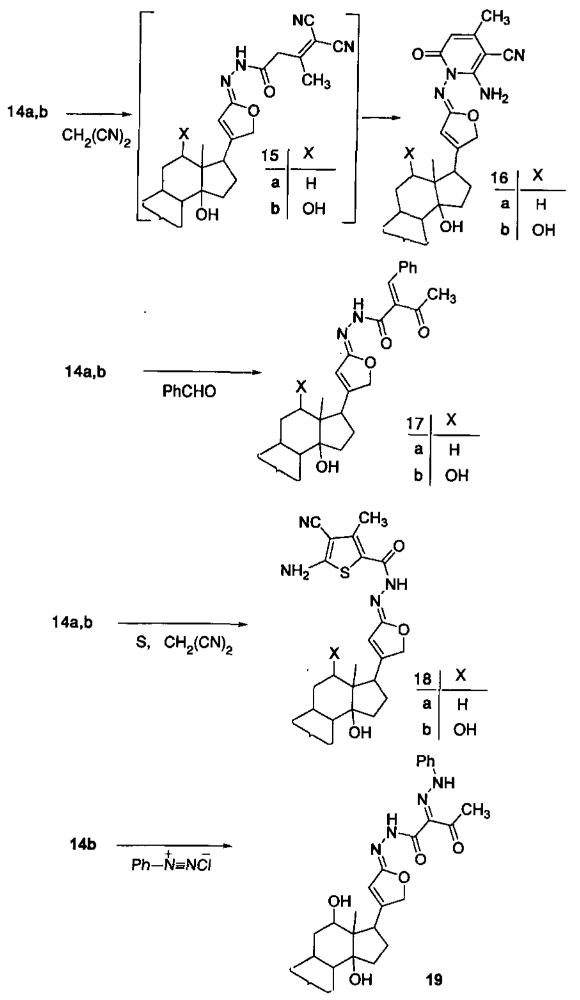
To a solution of **14a** (0.86 g, 0.001 mol) or **14b** (0.87 g, 0.001 mol) in ethanol (20 ml) containing piperidine (0.5 ml), benzaldehyde (0.1 g, 0.001 mol) was added. The reaction mixture was heaed under reflux for 4h then left to cool. The solid product formed upon dilution with water was collected by filtration.

Compound **17a**: Orange crystals from ethanol, yield 79%, m.p. 138-140°C. IR: 3400-3200 (OH, NH), 3000 (aromatic CH), 2980, 2890 (CH_3, CH_3), 1750 (C=O), 1650-1630 cm^{-1} (C=N, C=C). ^1H NMR (δ ppm): 2.51 (s, 3H, CH_3), 7.40 (s, 1H, CH =C), 7.33-7.40 (m, 5H, C_6H_5), 7.98 (s, 1H, NH). $C_{52}H_{74}O_{15}N_2$: Calcd: C, 65.68 H, 7.78 N, 2.94. Found: C, 65.48 H, 7.75 N, 2.75

Compound **17b**: Orange crystals from ethanol yield, 79%, m.p. 138-140°C. IR: 3400-3200 (OH , NH), 3000 (CH aromatic), 2980, 2890 (CH_3, CH_2), 1750 (C=O), 1645-1630 cm^{-1} (C=N, C=C). ^1H NMR (δ ppm): 2.51 (s, 3H , CH_3), 7.23 (s, 1H, CH =C), 7.33-7.40 (m, 5H, C_6H_5), 7.98 (s, 1H, NH). $C_{52}H_{74}O_{15}N_2$: Calcd: C, 64.59 H, 7.66 N, 2.89. Found: C, 64.35 H, 7.45 N, 2.64.

22-[2-amino-3-cyano-4-methyl-5-carbohydrazido-thiopheno]digitoxin (18a) and 22-[2-amino-3-cyano-4-methyl-5-carbohydrazido-thio-pheno]digoxin (18b)

To a solution of **14a** (0.86 g, 0.001 mol) or **14b** (0.87 g, 0.001 mol) in ethanol (20 ml) containing triethylamine (0.5 ml), elemental sulfur (0.03 g, 0.001 mol) was added followed by malononitrile (0.6 g, 0.001 mol). The reaction mixture was heated under reflux for 3 h then poured into ice/water containing a few drops of hydrochloric acid and the formed solid product was collected by filtration.



Scheme 4. Synthesis of compound **16**, **17**, **18** and **19**

Compound **18a** : Brown crystals from methanol, yield 80%, m.p. 220°C. IR: 3400-3200 (OH, NH, NH_2), 2980, 2890 (CH_3, CH_2), 2200 (CN), 1750 (C=O), 1645-1630 cm^{-1} (C=N, C=C). ^1H NMR (δ ppm): 2.61 (s, 3H, CH_3), 3.76 (s, 2H, NH_2), 7.46 (s, 1H, NH). $C_{48}H_{70}O_{13}N_4S$: Calcd: C, 61.14 H, 7.43 N, 5.94 S, 3.39. Found: C, 61.01 H, 7.37 N, 5.89 S, 3.25.

Compound **18b** : Brown crystals from ethanol, yield 79 %, m.p. 140°C. IR: 3400-3200 (OH, NH, NH_2), 2980, 2890 (CH_3, CH_2), 2200 (CN), 1750 (C=O), 1645-1630 cm^{-1} (C=N , C=C). ^1H NMR (δ ppm): 2.61 (s, 3H, CH_3), 3.76 (s, 2H, NH_2), 7.46 (s, 1H, NH). $C_{48}H_{70}O_{14}N_4S$: Calcd: C, 60.12 H, 7.30 N, 5.84 S, 3.34. Found: C, 60.01 H, 7.0 N, 5.71 S, 3.15.

22-[α-acetyl-α-phenylhydrazonoacetic acid hydrazono] digoxin (19)

To a cold solution of **14b** (0.87 g, 0.001 mol) at 0°C in ethanol (20 ml) containing sodium acetate (0.08 g , 0.001

mol), a solution of benzendiazonium chloride [prepared by the addition of Sodium nitrite solution (0.06 g, 0.001 mol) to a cold suspension of aniline (0.09 ml, 0.001 mol) containing the appropriate amount of hydrochloric acid with stirring] was added. The solid product formed upon dilution with water was collected by filtration.

Compound **19**: Brown crystals from dioxan, yileld 82 %, m.p. 93-95°C. IR : 3400-3200 (OH, NH), 3000 (C_6H_5), 2980, 2890 (CH_3, CH_2), 1750 (C=O), 1660-1630 cm^{-1} (C=N, C=C). ^1H NMR (δ ppm): 2.50 (s, 3H, CH_3), 7.36-7.40 (m, 5H, C_6H_5), 7.62-7.65 (2s, 2H, 2NH). $C_{51}H_{74}O_{15}N_4$: Calcd.: C, 62.32 H, 7.53 N, 5.70. Found: C, 62.1 H, 7.2 N, 5.73.

22-[*N*-imino-4-methyl-3-cyano-6-hydroxy-2-oxopyridino) digoxin (20)

To a solution of **14b** (0.87 g, 0.001 mol) in ethanol (20 ml) containing piperidine (0.5 ml), ethyl cyanoacetate (0.1 g, 0.001 mol) was added. The reaction mixture heated under reflux for 4h then poured into ice/water containing few drops of hydrochloric acid and the formed solid product was collected by filtration,

Compound **20**: Yellow crystals from 1,4-dioxane, yield 82%, m.p. 138-140°C. IR: 3400-3200 (OH, NH), 2980, 2890 (CH_3, CH_2), 2200 (CN), 1750 (C=O), 1645-1630 cm^{-1} (C=N, C=C). ^{1}HNMR (δ ppm): 2.89 (s, 3H, CH_3), 7.03 (s, 1H, pyridine H-3), 10.45 (s, 1H, OH). $C_{48}H_{69}O_{15}N_3$: Calcd C, 62.13 H, 7.44 N, 4.5. Found: C, 62.01 H, 7.21 N, 4.3.

ω-Bromoacetoacetic acid hydrazido digoxin (21)

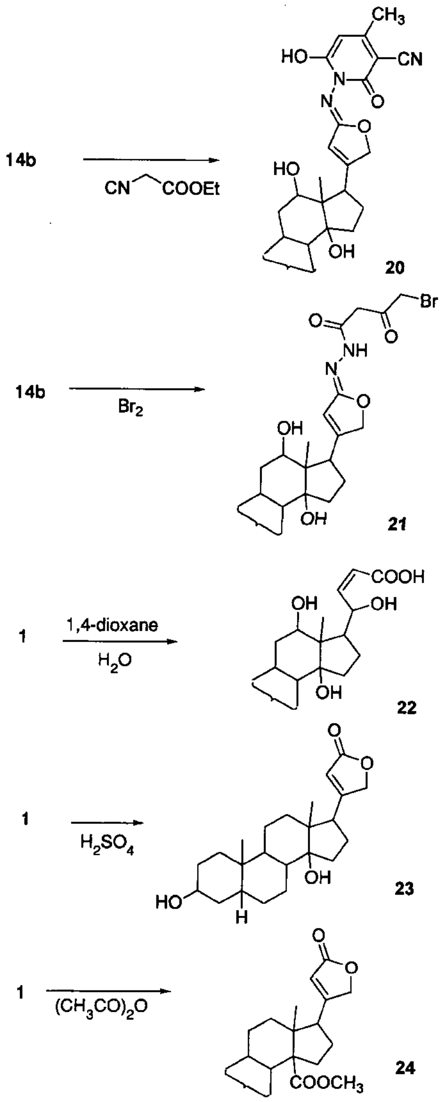
To a hot solution (at 50°C) of **14b** (0.87 g, 0.001 mol) in the less amount of acetic acid, bromine (0. 08 g, 0.001 mol) was added with stirring then left to cool. The reaction mixture poured into ice/water the solid product formed was collected by filtration.

Compound **21**: White crystals from methanol yield, 78%, m.p. 180°C. IR: 3480-3360 (NH), 2890 (CH2), 1750-1720 (2C=O), 1645-1630 cm^{-1} (C=N, C=C). ^1H NMR (δ ppm): 4.89, 5.58 (2s, 4H, $2NH_2$), 9.12 (s, 1H, NH). $C_{45}H_{69}O_{15}N_2Br$): Calcd: C, 56.42 H, 7.21 N, 2.92 Found: C, 56.23 H, 7.0 N, 2.81.

Digitoxin-α-methyl-p-hydroxypropenoic acid (22)

To a solution of digitoxin (0.76 g, 0.001 mol) in 1,4-dioxane (30 ml) containing water (3 ml) was added. The reaction mixture was heated under reflux on a boiling water bath for 4 h then left to cool. The solid product fromed was collected by filtration.

Compound **22**: White crystals from ethanol, yield 85 %, m.p. 138°C. IR: 3580-3320 (2OH), 2950, 2890 (CH_3, CH_2), 1750 (C=O),1635 cm^{-1} (C=C). ^1H NMR (δ ppm): 5.31 (s, 2H , CH_2), 6.78 (s, 1H, CH=C), 9.32, 10.25 (2s, 2H, 2OH). $C_{41}H_{66}O_{14}$: Calcd C, 62.91 H, 8.43. Found: C, 62.75 H, 8.39.



Scheme 5. Synthesis of compound **20~24**

Digitoxigenin (23)

To a solution of digitoxin (0.76 g, 0.001 mol) in methanol (20 ml), sulphuric acid (0.5 ml) was added then the reaction mixture was heated under reflux for lh on water bath then left to cool. The solid product fromed was collected by filtration.

Compound **23**: White crystals from methanol, yield 82 %, m.p. 245-248°C. IR: 3480-3200 (OH), 2980, 2890 (CH_3, CH_2), 1760 (C=O), 1645-1630 cm^{-1} (C=N, C=C). ^1H NMR (δ ppm): 1.16 , 1.17 (2s, 6H, $2CH_3$), 2.25- 2.35 (m, 18H, $9CH_2$), 2.45-2.56 (s, 5H, 5CH), 4.58 (s, 2 H, furan CH_2), 6.0 (s, 1H, furan CH). $C_{23}H_{34}O_4$: Calcd: C, 73.79 H, 9.09. Found: C, 73.75 H, 9.02

Digitoxin 14-acetate (24)

To a solution of digitoxin (0.76 g, 0.001 mol) in methanol (20 ml) containing anhydrous sodium acetate (0.8 g, 0.001 mol), acetic anhydride (0.102 g, 0.001 mol) was added. The reaction mixture was heated in water both for 3h and the solid product formed was collected by filtration.

Compound **24**: White crystals from methanol, yield 82%, m.p. 148-150°C. IR: 3200 (OH), 2980, 2890 (CH_3, CH_2), 1750 (C=O), 1630 cm^{-1} (C=C). ^1H NMR (δ ppm): 2.27 (s, 3H, CH_3). $C_{43}H_{66}O_{14}$: Calcd: C, 64.01 H, 8.13. Found: C, 64.02 H, 8.15.

RESULTS AND DISCUSSION

Both of digitoxin **1** and digoxin **2** react with cyanoacetic acid hydrazide to produce the hydrazone derivatives **3a,b**. The structures of the latter products were established on the basis of analytical and spectral data. Thus, IR spectrum of **3a** showed the presence of an NH group stretching at υ 3300 cm^{-1}, one OH group stretching at υ 3200 cm^{-1}, one C=O group stretching at υ 1700 cm^{-1} and one CN group stretching at υ 2225 cm^{-1}. Moreover, the ^1H NMR spectrum showed the presence of a singlet (D_2O exchangeable) at δ 7.76 for NH, a singlet at δ 4.98 for CH_2. Further confirmation for the structures of **3a** and **3b** is obtained through studying their reactivity towards chemical reagents. Thus, the reaction of **3a,b** with benzenediazonium chloride at 0-5°C gave the phenyl hydrazone derivatives **4a** and **4b** respectively. The structures of the latter products were based on analytical and spectral data. Thus, the IR spectrum of **4a** showed the presence of an NH stretching at υ 3350 cm^{-1}, one C=O group stretching at υ 1700 cm^{-1}, one CN group stretching at 2225 cm^{-1}. Moreover, the ^1H NMR spectrum showed the presence of a singlet at δ 6.50 and 7.0 for two NH group (D_2O exchangeable), and a mutiplet at δ 7.50-7.89 for phenyl group.

The reaction of either **3a** or **3b** with salicylaldehyde gave the coumarin derivatives **6a** and **6b** respectively. Formation of the latter products is assumed on the basis of the first formation of the arylidene derivatives **5a,b** followed by cyclization and hydrolysis (Gewald *et al.*, 1990, 1980; Sherif et *al.*, 1996) of the imino group into keto to afford the final inseparable coumarin derivatives **6a,b**. The structures of **6a** and **6b** was confirmed on the basis of analytical and spectral data (see experimental section).

Compounds **3a,b** showed the usual activity of cyanomethylene derivatives. Thus, the reaction of either **3a** or **3b** with sulfur and malononitrile in ethanolic triethylamine solution as a type of Gewalds reaction (Selim et *al.*, 1998); Gewald et *al.*, 1988; Mohareb et *al.*, 1994) gave the thiophene derivatives **7a** and **7b**. Structures of the latter products were confirmed on the basis of analytical and spectral data. Thus, the IR spectra of **7a** and **7b** showed the presence on NH_2, NH stretchings at υ 3345-3350 cm^{-1}, OH stretching at υ 3300 cm^{-1}, one C=O group stretching at υ 1750 cm^{-1} and CN group stretching at υ 2225 cm^{-1}. Moreover, the ^1H NMR spectrum showed the presence of two singlets at δ 4.77, 5.70 for two NH_2 groups (D_2O exchangeable), a singlet at δ 8.46 for two NH groups (D_2O exchangeable). Moreover, the reaction of **3a** and **3b** with sulfur and phenyl isothiocyanate (Mohareb et *al.*, 1994; Gewald et *al.*, 1989) afforded the thiazole derivatives **8a** and **8b**. Structures of the latter products were based on ^1H NMR spectra which revealed in case of **8a** and **8b** a singlet at δ 4.48 for NH_2, a multiplet at δ 7.56 for C_6H_5 and a singlet at δ 9.75 for NH.

The reactivity of **3a** and **3b** towards cyanomethylene reagents was studied to give pyridine and coumarin derivatives with potential biological activities (Antonello et *al.*, 1979). Therefore, the reaction of either **3a** or **3b** with malononitrile gave the pyridine adducts **10a** and **10b** respectively. The IR spectrum of **10a** showed one cyano group stretching at υ 2220 cm^{-1}. Moreover, the ^1H NMR of **10a** showed two singlets at δ 4.89 and 4.59 (D_2O exchangeable) for two NH_2 groups, and a singlet at δ 5.75 for pyridine H-5 proton. Formation of **10a** and **10b** was explained in terms of the intermediate formation of **9a** and **9b** respectively. Especially compound **3a** reacted with sulfur and ethyl cyanoacetate as a type of Gewald's reaction to afford the thiophene derivative **11**.

The reaction of digitoxin and digoxin with hydrazine hydrate gave the hydrazone derivatives **12a** and **12b** respectively. The reaction of the latter products with malononitrile gave the acyclic adducts **13a** and **13b** respectively. The structures of **13a** and **13b** were confirmed on the basis of analytical and spectral data. Thus, the IR spectra of **13a,b** showed the presence of NH_2 stretching at υ 3400 cm^{-1}, OH group stretching at υ 3200 cm^{-1} and one CN group stretching at υ 2225 cm^{-1}. Moreover, the ^1H NMR spectrum showed the presence of a singlet at δ 3.60 for NH_2 and a singlet at δ 3.50 for CH_2. The Reaction of **12a** and **12b** with ethyl cyanoacetate gave the same hydrazone derivatives **3a** and **3b** obtained before (mixed m.p. and finger print IR). The reaction of **12a** and **12b** with ethyl acetoacetate gave the hydrazide derivatives **14a** and **14b**. The IR and ^1H NMR spectra are in agree in all respects with the proposed structures. Further confirmations for these structures were obtained by studying their chemical reactivity with some chemical reagents. Thus, the reaction of **14a** and **14b** with malononitrile gave the pyridine derivatives **16a** and **16b**. formation of the latter products took place via the intermediate formation of **15a** and **15b**. Moreover, the reaction of **14a** and **14b** with benzaldehyde yielded the benzal derivatives **17a** and **17b** respectively. On the other hand, the reaction of **14a** and **14b** with sulfur and malononitrile gave the thiophene derivatives **18a** and **18b**.

The reaction of **14b** with benzenediazonium chloride gave the phenylhydrazone derivative **19**. On the other hand, the reaction of **14b** with ethyl cyanoacetate gave the pyridine derivatives **20**. Structures of compounds **19** and **20** were based on analytical and spectral data (see experimental data).

The acetyl group present in compound **14b** found to *be highly reactive towards electrophilic reagents.* Thus with bromine in glacial acetic acid, compound **14b** gave the monobromo derivative **21**.

Our trials to hydrolyse digitoxin to get a water-soluble product were successful. Thus, the reaction of digitoxin (**1**) with 1,4-dioxane containing water afforded the unsaturated acid **22**. On the other hand, the use of concentrated sulfuric acid with **1** yielded **23**. Actylation of digitoxin (**1**) in sodium acetate/acetic anhydride mixture gave the acetyl derivative **24**.

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