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Digoxin derivatives substituted by alkylidene at the butenolide part

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ABSTRACT

A series of digoxin derivatives containing the γ-alkylidene butenolide moiety were synthesised by way of stereoselective vinylogous aldol reaction of the unactivated butenolide in simple conditions. The structures of compounds synthesised were characterised by infrared (IR), nuclear magnetic resonance (NMR) and HR-MS. Preliminary bioassay shows that some of them have cardiac functions, especially compound **2g** that induced a marked increase in myocardial contractility at 10 ng ml^{-1} and 20 ng ml^{-1} concentrations without digitalis toxicity.

Plants have been a rich source of bioactive glycosides. The digitalis family, in particular, has produced a number of steroidal glycosides, which have been used medicinally in the treatment of cardiac diseases for centuries [1]. Digoxin (Fig. 1) is one of the constituents of digitalis, which has been widely used medicinally in the treatment of cardiac diseases for centuries and is still used in the management of various cardiac arrythmias. [2] It was the second most frequently prescribed drug in the US [3]. Unfortunately, digoxin has a low therapeutic index and can cause toxic effects, such as arrhythmias and ventricular fibrillations. It is necessary to measure serum concentrations of the drug to maintain proper doping [2].

Clearly, understanding the structural features of complex formation between digoxin and its receptor, Na^+, K^+-ATPase, could lead to the development of inotropic drugs with higher therapeutic indices. Therefore, the synthesis and the structure–activity relationship study of digoxin analogues also led drug research chemists to take interest [2,4–8]. Farr et al. [7] developed a three-dimensional quantitative structure–activity relationship (3D-QSAR) model for the inhibition of Na^+, K^+-ATPase by cardiotonic steroids using comparative molecular field analysis (CoMFA) (Fig. 2). The contour maps visualise the areas of the steric and electrostatic fields that are the most important for explaining the variation in enzyme inhibition by the series of compounds. The green regions near the lactone ring indicate where an increased steric bulk in the steric contour map increases the ability of the ligand to inhibit the enzyme (Fig. 2(A)). The red regions in the electrostatic (lower) contour map (Fig. 2(B)), indicate that an increased electronegativity around the lactone ring ought to increase inhibition. In addition, studies have shown that the derivatives of cardiac glycosides containing γ-substituted butenolide can influence the Na^+, K^+ channels [9]. Digoxin substituted at the butenolide moiety probably regulates the intercellular Na^+, K^+ concentrations.

However, there were no reports about digoxin derivative substituted at the butenolide moiety [9,10]. Here, a simple and efficient method of derivatising the butenolide ring of digoxin is described. The butenolide ring was substituted by a reaction with aldehyde. Usually, the reaction requires a strong base, low temperature and several steps, especially the hydroxyls in the substrate must be protected. Here, a series of γ-substituted digoxin derivatives were synthesised from digoxin in one pot in a simple manner. At the same time, a preliminary bioassay regarding the effects on the isolated guinea pig heart was also carried out.

1. Experiment

1.1. General comments

Infrared (IR) spectra were recorded as KBr pellets on a Thermo Nicolet (IR200) spectrometer. All mass spectrometry experiments were performed on a Waters Q-T of micro mass spectrometer equipped. ^1H and ^{13}C spectra were recorded respectively at 400 and 100 Hz with a Brüker DPX-400 spectrometer with TMS (tetramethylsilane) as the internal standard. Melting points (m.p.) were determined on a (2F-2) apparatus.

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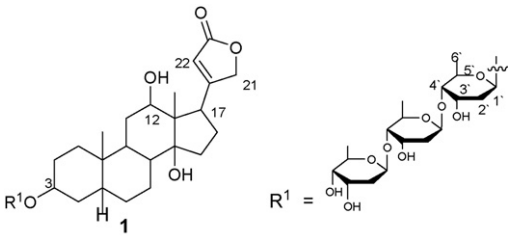


Fig. 1. Digoxin.

1.2. Synthesis of 21-aromaticidene digoxin

A catalyst, anhydrous Na_2CO_3, was added to the solution of digoxin (0.06 mmol) and aldehyde (0.18–0.5 mmol) in methanol (6 ml). The reaction mixture was stirred in refluxing for 6 h and then diluted with ethyl acetate and washed by aqueous NaCl and water. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was separated by column chromatography to yield the compound **2** or **4**.

1.2.1. 21-(2-Furanmethanylidene)-digoxin (**2a**)

Yield 95%; m.p. 172.8–173.8 °C; IR 3462, 2933, 2880, 1742, 1645, 1472, 1449, 1405, 1380, 1274, 1163, 1128, 1068, 1015, 953, 869 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 7.74 (1H, d, J = 1.3 Hz, OCH=), 7.01 (1H, d, J = 3.2 Hz, C=CH), 6.64 (1H, s, H-24), 6.51 (1H, dd, J = 1.6, 3.2 Hz, C=CH–CH=), 6.17 (1H, s, H-22), 4.87–4.92 (3H, m, 3 × H-1′), 4.2 (2H, t, J = 3.7 Hz), 4.1 (1H, m), 4.03 (1H, br), 3.75–3.78 (3H, m), 3.48 (1H, m), 3.75 (1H, m), 3.2–3.3 (4H, m), 2.25 (1H, m, H-17), 0.91 (3H, s, CH_3-19), 0.77 (3H, s, CH_3-18); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 170.2, 164.4, 149.5, 148.4, 143.8, 115.6, 114.9, 112.9, 100.1, 98.4, 98.3, 95.5, 86.5, 82.6, 28.2, 75.7, 72.7, 72.6, 69.6, 68.3, 68.1, 66.5, 66.4, 55.5, 42.1, 41.5, 37.8, 37.1, 36.7, 36.3, 35.0, 32.9, 32.6, 30.6, 29.8, 29.5, 26.6, 26.5, 23.6, 21.7, 18.2, 8.8; HR-MS mz^{-1} $[M+Na]^+$ 881.4330 (calculated 881.4300), calculated for $C_{46}H_{66}NaO_{15}$.

1.2.2. 21-Benzylidene digoxin (**2b**)

Yield 94%; m.p. 167.6–168.5 °C; IR 3437, 2932, 2880, 1741, 1696, 1639, 1450, 1404, 1380, 1317, 1272, 1164, 1128, 1068, 1014, 954, 869 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, TMS): δ 7.80 (2H, d, J = 7.2 Hz, Ar-H), 7.41 (3H, m, Ar-H), 6.6 (1H, s, H-24), 6.20 (1H, s, H-22), 4.90–4.24 (3H, m, 3 × H-1″), 4.24 (2H, br), 4.11 (1H, s), 4.03 (1H, s), 3.82–3.77 (3H, m), 3.53 (2H, m), 3.30–3.19 (4H, m), 3.0 (2H, br), 2.30 (1H, m, H-17), 0.91 (3H, s, CH_3-19), 0.78 (3H, s, CH_3-18); ^{13}C NMR (100.6 MHz, $CDCl_3$, TMS): δ 170.5, 165.2, 150.1, 133.2, 130.7, 129.0, 128.6, 128.3, 115.6, 111.2, 98.2, 98.1, 95.3, 86.5, 82.4, 82.1, 75.7, 72.6, 72.4, 69.4, 68.2, 68.0, 66.3, 66.2, 55.3, 42.0, 41.4, 37.7, 37.0, 36.6, 36.1, 34.9, 32.8, 32.5, 30.6, 30.1, 29.7, 29.4, 26.5, 26.4, 23.5, 18.1, 8.7; HR-MS mz^{-1}: $[M+Na]^+$ 891.4518 (calculated 891.4507), calculated for $C_{48}H_{68}NaO_{14}$.

1.2.3. 21-p-Fluorobenzylidene digoxin (**2c**)

Yield 95%; m.p. 168.4–169.6 °C; IR 3453, 2933, 2876, 1746, 1602, 1508, 1449, 1406, 1380, 1316, 1232, 1163, 1068, 1014, 954, 869 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, TMS):δ 7.8 (2H, dd, J = 6.0, 8.4 Hz H, Ar-H), 7.08 (2H, t, J = 8.4 Hz, Ar-H), 6.58 (1H, s, H-24), 6.20 (1H, s, H-22), 4.92–4.85 (3H, m, 3 × H-1′), 4.25 (2H, s), 4.13 (1H, d, J = 2.8 Hz), 4.04 (1H, s), 3.79–3.72 (4H, m), 3.50 (2H, m), 3.25–3.22 (4H, m), 2.25 (1H, m), 0.92 (3H, s, CH_3-19), 0.78 (3H, s, CH_3-18); ^{13}C NMR (100.6 MHz, $CDCl_3$, TMS): δ 170.3, 165.1, 164.1, 161.5, 149.7, 132.6, 132.5, 129.6, 115.8, 115.6, 109.9, 98.2, 98.1, 95.3, 86.5, 28.5, 82.1, 76.3, 75.8, 72.6, 72.4, 69.5, 68.2, 68.0, 66.4, 66.3, 55.3, 42.1, 41.5, 37.7, 37.0, 36.6, 36.2, 34.9, 32.8, 32.6, 29.7, 29.5, 26.6, 26.4, 23.5, 21.6, 18.4, 18.1, 8.7; HR-MS mz^{-1}: $[M+Na]^+$ 909.4405(calculated.909.4413), calculated for $C_{48}H_{67}FNaO_{14}$.

1.2.4. 21-m-Bromophenylidene digoxin (**2d**)

Yield 80%; m.p. 158.4–160.1 °C; IR 3442, 2932, 2876, 1748, 1638, 1590, 1449, 1405, 1380, 1316, 1273, 1164, 1128, 1069, 1014, 954, 868 cm^{-1}; ^1H NMR (400 MHz, dimethylsulphoxide (DMSO), TMS):δ 7.83 (1H, s, Ar-H), 7.66 (1H, d, J = 7.2 Hz, Ar-H), 7.51 (1H, br), 7.42 (1H, d, J = 7.2 Hz, Ar-H), 6.73 (1H, s, H-24), 6.24 (1H, s, H-22), 5.01 (3H, m), 4.04 (3H, br), 3.90 (1H, br), 3.84 (1H, s), 3.69–3.63 (4H, m), 3.13 (3H, m), 3.00 (1H, br), 2.13 (1H, m, H-17), 0.84 (3H, s, CH_3-19), 0.64 (3H, s, CH_3-18); ^{13}C NMR (100.6 MHz, DMSO, TMS): δ 169.2, 166.7, 150.9, 135.7, 132.3, 131.5, 131.3, 131.2, 129.2, 122.3, 115.7, 108.6, 99.3, 99.2, 95.5, 85.2, 82.1, 81.8, 73.9, 72.8, 72.3, 69.2, 67.8, 67.7, 67.2, 66.5, 66.3, 60.0, 56.0, 42.1, 40.6, 38.6, 38.5, 38.1, 36.5, 34.9, 32.5, 31.9, 30.3, 30.1, 29.8, 29.1, 26.6, 26.2, 23.8, 18.5, 18.2, 9.6; HR-MS mz^{-1}: $[M+Na]^+$ 969.3649 (calculated 969.3612), $[M+2+Na]^+$ 971.3662, calculated for $C_{48}H_{67}BrNaO_{14}$.

1.2.5. 21-p-Chlorobenzylidene digoxin (**2e**)

Yield 92%; m.p. 167.1–168.1 °C; IR 3467, 2931, 2880, 1746, 1697, 1646, 1592, 1490, 1449, 1408, 1380, 1315, 1273, 1164, 1068, 1017, 953, 869, 826, 754, 730 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, TMS):δ 7.72 (2H, d, J = 8.0 Hz, Ar-H), 7.34 (2H, d, J = 8.0 Hz, Ar-H), 6.55 (1H, s, H-24), 6.21 (1H, s, H-22), 4.91–4.85(3H, m, 3 × H-1′), 4.24 (2H, br), 4.12 (1H, m), 4.03 (1H, s), 3.81–3.75 (3H, m), 3.44–3.47 (2H, m), 3.30 (1H, d), 3.25–3.21 (3H, m), 3.1 (2H, br), 2.5 (1H, br), 2.21 (1H, m, H-17), 0.92 (3H, s, CH_3-19), 0.78 (3H, s, CH_3-18); ^{13}C NMR (100.6 MHz, $CDCl_3$, TMS): δ 170.4, 165.4, 150.8, 135.0, 132.2, 129.3, 116.4, 110.1, 98.68, 98.62, 95.8, 95.8, 86.9, 82.9, 82.6, 76.2, 73.1, 72.8, 69.9, 68.6, 68.5, 66.8, 66.7, 55.83, 42.5, 41.9, 38.2, 37.5, 37.1, 36.6, 35.4, 33.3, 33.0, 31.1, 30.6, 30.2, 30.1, 29.9, 27.0, 26.8, 23.9, 22.0, 18.5, 9.1; HR-MS mz^{-1}: $[M+Na]^+$ 925.4136 (calculated 925.4117), calculated for $C_{48}H_{67}ClNaO_{14}$.

1.2.6. 21-o-Chlorobenzylidenedigoxin (**2f**)

Yield 79%; m.p. 166.5–167.4 °C; IR 3472, 2933, 1884, 1747, 1594, 1448, 1405, 1380, 1317, 1272, 1164, 1128, 1068, 1013, 953, 869, 755, 731 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, TMS):δ 8.24 (1H, d, J = 7.6 Hz, Ar-H), 7.36 (2H, t, J = 7.2 Hz, Ar-H), 7.06 (1H, s, H-24), 7.02 (1H, d, J = 6.8 Hz, Ar-H), 6.25 (1H, s, H-22), 4.90–4.84 (3H, m, 3 × H-1′), 4.24 (2H, br), 4.12 (1H, br), 4.03 (1H, s), 3.77–3.75 (4H, m), 3.44–3.47 (2H, m), 3.29–3.19 (3H, m), 3.1 (2H, br), 2.27 (1H, m, H-17), 0.92 (3H, s, CH_3-19), 0.78 (3H, s, CH_3-19); HR-MS mz^{-1}: $[M+Na]^+$ 925.4127 (calculated. 925.4117), calculated for $C_{48}H_{67}ClNaO_{14}$.

1.2.7. 21-m-Chlorobenzylidene digoxin (**2g**)

Yield 96%; m.p. 171.6–172.5 °C; IR 3453, 2932, 2883, 1748, 1679, 1648, 1594, 1449, 1406, 1380, 1273, 1165, 1128, 1068, 1013, 954, 907, 868, 755, 730 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, TMS): 7.74 (1H, s, Ar-H), 7.69 (1H, d, J = 7.2 Hz, Ar-H), 7.29 (2H, br, Ar-H), 6.54 (1H, s, H-24), 6.22 (1H, s, H-22), 4.91 (3H, m), 4.24 (2H, s), 4.12 (1H, m), 4.02

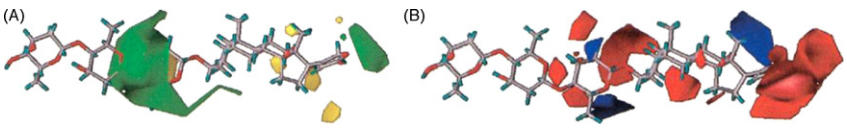


Fig. 2. 3D-QSAR map for Na^+, K^+-ATPase by cardiotonic steroids using CoMFA.

(1H, s), 3.82–3.77 (3H, m), 3.53 (2H, m), 3.30 (1H, d, J = 8.0 Hz), 3.25 (2H, m), 3.0 (2H, br) 2.27 (1H, m, H-17), 0.92 (3H, s, CH_3-19), 0.78 (3H, s, CH_3-18); ^{13}C NMR (100.6 MHz, $CDCl_3$, TMS): δ 169.9, 165.1, 150.9, 135.1, 134.5, 130.3, 129.8, 128.7, 128.6, 116.4, 109.5, 98.3, 98.2, 95.4, 86.6, 82.5, 82.2, 75.8, 72.7, 72.5, 69.5, 68.2, 68.0, 66.4, 66.3, 55.4, 42.1, 41.5, 37.8, 17.1, 36.7, 36.2, 35.0, 32.9, 32.6, 30.7, 30.2, 29.8, 29.5, 26.6, 26.4, 23.5, 18.1, 8.7; HR-MS mz^{-1}: [M+Na]$^+$ 925.4111(calculated 925.4117), calculated for $C_{48}H_{67}ClNaO_{14}$.

*1.2.8. 21-p-Methoxylbenzylidene digoxin (**2h**)*

Yield 93%; m.p. 156.5–157.8 °C; IR 3455, 2932, 2880, 1737, 1604, 1511, 1449, 1406, 1379, 1302, 1255, 1175, 1128, 1061, 1014, 954, 868, 829 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): 7.75 (2H, d, J = 8.4 Hz, Ar-H), 6.89 (2H, d, J = 8.6 Hz, Ar-H), 6.6 (1H, s, H-24), 6.15 (1H, s, H-22), 4.85–4.92 (4H, m),4.24 (2H, br), 4.11 (1H, s), 4.02 (1H,s), 3.84 (3H, s, OCH_3), 3.75–3.82 (3H, m), 3.49 (2H, m), 3.2–3.15 (4H, m), 0.92 (3H, s, CH_3-19), 0.78 (3H, s, CH_3-18); ^{13}C NMR (100.6 MHz, $CDCl_3$, TMS): δ 170.8, 165.2, 160.1, 148.7, 132.5, 126.2, 114.6, 114.2, 111.3, 98.3, 98.2, 95.4, 86.5, 82.5, 82.1, 75.8, 72.6, 72.5, 69.5, 68.2, 67.9, 66.4, 66.3, 53.4, 55.3, 42.1, 41.5, 37.8, 37.1, 36.7, 36.2, 34.9, 32.8, 32.6, 29.7, 29.5, 26.6, 26.4, 23.5, 21.6, 18.1, 8.7; HR-MS mz^{-1}: [M+Na]$^+$921.4631 (calculated 921.4613), calculated for $C_{49}H_{70}NaO_{15}$.

*1.2.9. 21-m-Methoxylbenzylidene digoxin (**2i**)*

Yield 90%; m.p. 171.6–172.5 °C; IR 3466, 2933, 2880, 1739, 1597, 1487, 1450, 1405, 1379, 1305, 1247, 1164, 1129, 1067, 1014, 654, 869, 754 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, TMS):δ 8.20 (1H, d, J = 8.0 Hz, Ar-H), 7.30 (2H, m, Ar-H), 7.10 (1H, s, H-24), 6.86 (1H, d, J = 8.0 Hz, Ar-H), 6.19 (1H, s, H-22), 4.91–4.84 (3H, m, 3 × H-1′), 4.24 (3H, s), 4.12 (1H, s), 4.03 (1H, s), 3.85 (3H, s, OCH_3), 3.82–3.77 (4H, m), 3.57 (2H, m), 3.29–3.19 (4H, m), 2.27 (1H, m), 0.91 (3H, s, CH_3-19), 0.78 (3H, s, CH_3-18); ^{13}C NMR (100.6 MHz, $CDCl_3$, TMS): δ 170.7, 165.5, 157.6, 150.0, 131.5, 130.2, 122.2, 121.0, 115.2, 110.5, 105.0, 98.2, 98.1, 95.3, 86.5, 82.4, 82.0, 75.6, 72.5, 72.4, 69.4, 68.2, 68.0, 67.9, 66.3, 66.2, 55.8, 55.5, 41.9, 41.4, 37.7, 37.0, 36.6, 36.1, 34.9, 32.8, 32.5, 29.4, 26.5, 26.4, 23.4, 21.6, 18.1, 8.7; HR-MS mz^{-1}: [M+Na]$^+$ 921.4631 calculated 921.4613), calculated for $C_{49}H_{70}NaO_{15}$.

*1.2.10. 21-(2,4,5-Trimethoxyl benzylidene)-digoxin (**2j**)*

Yield 91%; m.p. 162.2–163.9 °C; IR 3447, 2933, 2884, 1740, 1638, 1578, 1506, 1451, 1420, 1380, 1332, 1268, 1164, 1128, 1068, 1012, 869 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ 7.25 (1H, s, Ar-H), 7.02 (1H, s, Ar-H), 6.5 (1H, s, H-24), 6.17 (1H, s, H-22), 4.87 (3H, m), 4.22 (2H, s), 4.09 (1H, s), 4.01 (1H, br), 3.87 (9H, od), 3.87–3.77 (3H, m), 3.65 (1H, s), 3.48 (2H, m), 3.28–3.17 (4H, m), 0.90 (3H, s, CH_3-19), 0.76 (3H, s, CH_3-18); ^{13}C NMR (100.6 MHz, $CDCl_3$, TMS): δ 170, 165, 153, 149, 138, 138.3, 128.9, 115, 111.1, 107.9, 98.2, 95.3, 86.5, 82.5, 82.1, 76.7, 75.9, 72.6, 72.4, 69.5, 68.2, 68.0, 66.4, 66.3, 60.9, 56.2, 55.4, 42.1, 41.4, 37.7, 37.0, 36.6, 36.2, 34.9, 32.6, 29.7, 26.5, 26.4, 23.5, 18.1, 8.7; HR-MS mz^{-1}: [M+Na]$^+$ 981.4858 (calculated 981.4824), calculated for $C_{51}H_{74}NaO_{17}$.

1.3. 21-(1,3-Benzodioxol-5-ylmethylidene)-digoxin (2k)

Yield 86%; m.p. 152.5–153.7 °C; IR 3462, 2933, 2880, 1742, 1645, 1472, 1449, 1405, 1380, 1317, 1274, 1229, 1163, 1128, 1068, 1015, 953, 869 cm^{-1}; ^{1}H NMR (400 MHz, DMSO, TMS): δ 7.29 (1H, s, Ar-H), 7.12 (1H, d, J = 8.0 Hz, Ar-H), 7.02(1H, d, J = 8.0 Hz,, Ar-H), 6.69 (1H, s, H-24), 6.13 (1H, s, H-22), 6.09 (2H, s,O-CH_2-O), 5.0 (1H,br, OH). 4.8 (3H, ot, 3 × H-1′), 4.25 (2H, br, OH), 4.04 (2H, s), 3.91 (1H, s), 3.84 (1H, d, J = 2.4 Hz), 3.7–3.63(5H, m), 3.13 (3H, d, J = 9.2 Hz), 2.99 (1H, d), 2.23 (1H, m), 0.84 (3H, s, CH_3-19), 0.63 (3H, s, CH_3-18); ^{13}C NMR (100.6 MHz, DMSO, TMS): δ 169.5, 166.6, 152.9, 148.7, 148.1, 131.7, 128.8, 127.5, 125.9, 114.1, 109.2, 109.0, 10.1, 99.2, 99.1, 95.5, 85.1, 82.1, 81.8, 73.9, 72.8, 69.25, 67.8, 67.6, 67.2, 66.4, 66.3, 55.9, 41.1, 40.6, 38.6, 38.5, 38.1, 34.9, 31.8, 30.3, 30.1, 29.7, 29.2, 26.6, 26.2, 23.8, 18.5, 18.2, 9.6; HR-MS mz^{-1}: [M+Na]$^+$ 935.4416 (calculated 935.4405), calculated for $C_{49}H_{68}NaO_{16}$.

Table 1
Compounds synthesized.

Comps.	R^1	Comps.	R^1	Comps.	R^1
2a	Furoyl	**2e**	*p*-ClPh	**2i**	*m*-MeOPh
2b	Ph	**2f**	*o*-ClPh	**2j**	*2,4,5*-Trimeoph
2c	*p*-FPh	**2g**	*m*-ClPh	**2k**	benZo[1,3]dioxole-5-methanyl
2d	*m*-BrPh	**2h**	*p*-MeOPh	**2l**	$CH{=}C(CH_3)CH_2CH_2CH{=}C(CH_3)_2$

*1.3.1. 21-(3,7-Dimethyl-2,6-octanedieneylidene)-digoxin **(2l)***

Yield 94%; m.p.: 159.7–160.4 °C; IR 3453, 2966, 2933, 2880, 1734, 1635, 1578, 1449, 1405, 1378, 1316, 1273, 1164, 1128, 1068, 955, 869 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): 6.63 (1H, d, J = 11.2 Hz, H-24), 6.40 (1H, d, J = 10.4 Hz, C = C-CH_2), 6.08 (1H, s, H-22), 5.01 (1H, s, CH_2–C<u>H</u>=C–C), 4.90–4.75 (3H, m, 3 × H-1′), 4.25 (2H, br), 4.11 (1H, s), 4.02 (1H, s), 3.73–3.77 (6H, m), 3.44–3.47 (2H, m), 3.22–3.29 (5H, m), 3.10 (2H, br), 0.92 (3H, s, CH_3-19), 0.75 (3H, s, CH_3-18); ^{13}C NMR (100.6 MHz, $CDCl_3$, TMS): δ 170.5, 163.7, 149.1, 147.8, 123.7, 118.6, 115.0, 109.3, 98.3, 98.2, 95.4, 86.5, 82.5, 82.1, 72.6, 72.4, 69.5, 68.2, 68.0, 67.9, 66.4, 66.3, 57.0, 55.2, 42.0, 41.5, 40.1, 37.8, 37.1, 36.6, 36.2, 34.9, 32.8, 32.5, 31.5, 30.2, 28.9, 26.5, 25.7, 23.5, 21.6, 18.3, 18.1, 17.6, 17.1, 8.7; HR-MS mz^{-1}: [M+Na]$^+$ 937.5287 (calculated. 937.5290), calculated for $C_{51}H_{78}NaO_{14}$.

*1.3.2. 21,21-Dihydroxymethyl (**4**)*

m.p.: 184.3–185.5 °C; IR:3435, 2934, 2880, 1736, 1630, 1450, 1405, 1378, 1165, 1129, 1067, 1014, 869 cm^{-1}; ^{1}H NMR(400 Hz, $CDCl_3$): 5.87 (1H, s, H-22), 5.0 (1H, br), 4.81 (1H, br), 4.70–4.64 (3H, m), 4.55 (2H, br), 4.2 (1H, br), 4.15 (1H, br), 3.90 (4H,s, 2 × <u>CH_2</u>OH), 3.78 (2H, s), 3.73 (2H, s), 3.49-3.62 (5H, m), 3.01 (2H, d, J = 9.2 Hz), 2.89 (1H, br). 2.01 (1H, m), 0.72 (3H, s, CH_3-19), 0.54 (3H, s, CH_3-18); ^{13}C NMR (100 Hz, $CDCl_3$) 179.04, 173.29, 119.02, 99.34, 99.25, 95.54, 94.31, 84.52, 82.16, 81.9, 74.1, 72.89, 72.31, 70.09, 69.26, 67.82, 67.71, 67.23, 66.51, 66.36, 62.48, 59.43, 58.98, 55.71, 44.21,38.69, 38.53, 38.16, 36.54, 34.89, 32.96, 31.81, 30.21, 29.7, 26.7, 26.23, 21.7, 18.60, 18.26, 10.32; HR-MS mz^{-1}: [M+H]$^+$ 863.4398 (calculated 863.4405), calculated for $C_{43}H_{68}NaO_{16}$.

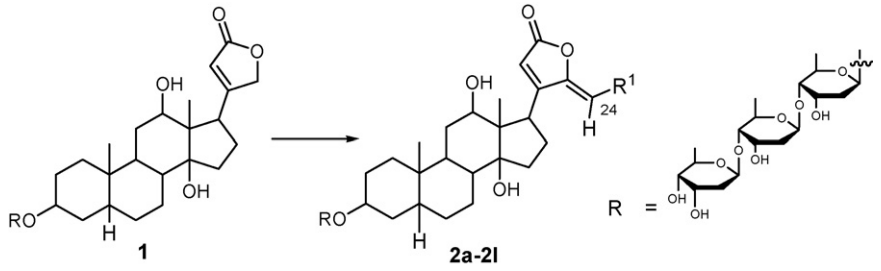
2. Results and discussion

The compound 21-alkylidene digoxin (**2**) was prepared by the reaction of digoxin with aldehyde in methanol in the presence of Na_2CO_3. Without strong base and functional protection, the reaction can proceed smoothly in refluxing (Scheme 1). Using this procedure, a series of 21-aromaticidene digoxin analogues (Table 1) were obtained with good yields in most cases. In the above reaction, aromatic aldehyde showed good reaction activity, but the reaction could not take place between the ketone or alkyl aldehyde and digoxin under the same conditions; this can be rationalised taking into consideration the electronic and steric factors.

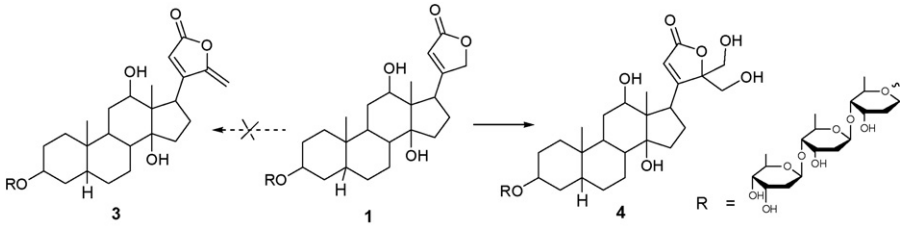
Reagent and conditions: aldehyde, Na_2CO_3, methanol, reflux, 6–12 h.

21-Methylidene digoxin (**3**) was designed by the reaction of digoxin with paraformaldehyde in the same condition. However, 21-dihydroxymethanyl digoxin (**4**) was obtained with a yield of 80% (Scheme 2). Namely, an addition reaction was carried out between digoxin and two formaldehyde molecules, and the dehydration reaction was inhibited.

Reagent and conditions: paraformaldehyde, Na_2CO_3, methanol, reflux, 6 h.



Scheme 1. Synthesis of compounds **2**.



Scheme 2. Synthesis of compound **4**.

The structures of compounds synthesised were characterised by NMR, IR and HR-MS. In ^{1}H NMR spectra of compounds **2a–2k**, a new singlet of double-bond proton was observed in about δ 6.5. Besides, the peak of Ar-H was also observed in about δ 6.5–8.2. In their ^{13}C NMR spectra, the corresponding unsaturated carbon signals were also observed. In the ^{1}H NMR spectra of compound **2l,** two doublet in δ 6.63 and δ 6.40 were observed. In the ^{1}H NMR spectra of compound **4**, four proton signals were observed in δ 3.90 and no double-bond proton signals though a double bond (Δ^{22}) was found. At the same time, the molecular formulas of the compounds synthesised were confirmed by HR-MS. The geometry of new generation double-bonds could not be confirmed by spectrum data, although compounds **2** could be confirmed to be single isomer by the ^{1}H NMR spectra.

According to the vinylogous aldol reaction, **5a–5d** should be the possible intermediate to yield the compounds **2** by an elimination reaction. To prove the geometry of the double bond (Δ^{24}) in compounds **2**, the three-dimensional conformation of intermediates **5a–5d** were shown in Fig. 3. According to the trans-coplanar principle for the elimination reaction, the conformations of intermediate **5a–5d** shown in Fig. 3 were necessary conditions. It could be found that an obvious steric hindrance exists between the phenyl group and steroid skeleton in intermediates **5b** and **5d**. On the contrary, the conformations of intermediates **5a** and **5c** favour the elimination reaction. Therefore, the conformations of **5a** and **5c** should be advantageous conformations. Based on the conformations of **5a** and **5c**, the geometry of the double bond (Δ^{24}) was deduced to be of the **Z** configuration. The above analysis could also rationalise why ketone could not react with digoxin under the same conditions.

Preliminary bioassay about the effects on the isolated guinea pig heart showed that some of the compounds **2** can increase myocardial contractility; especially, compound **2g** could make a marked increase in myocardial contractility in 10 ng ml^{-1} and 20 ng ml^{-1} without showing digitalis toxicity. Under the same conditions, digoxin showed significant digitalis toxicity. Further studies regarding the effects on the Na^+, K^+ ion channels are in progress.

In summary, a simple and efficient method of derivatising the butenolide ring of digoxin was described. A series of γ-substituted digoxin derivatives were synthesised from digoxin in one pot. A preliminary bioassay showed that some of them could increase myocardial contractility; especially, compound **2g** demonstrated

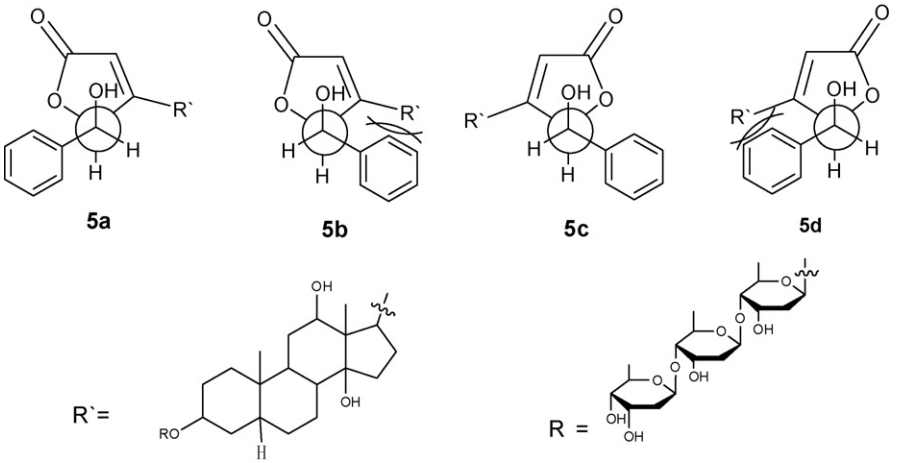


Fig. 3. The three-dimensional conformation of intermediate **5a**–**5d**.

a marked increase in myocardial contractility at 10 ng ml^{-1} and 20 ng ml^{-1} without digitalis toxicity. It is valuable to screen the new cardiac glycosides heart failure drug.

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