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Cholecystokinin-1 receptor antagonists: 5-hydroxy-5-aryl-pyrrol-2-ones as anticancer agents

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Supplementary Information

Table of Contents

1. Materials and Methods

1.1 Synthesis

The chemicals were obtained from Aldrich (Gillingham, UK) and Lancaster (Lancaster, UK). Atmospheric pressure chemical ionisation mass spectroscopy (APCI), negative or positive mode, was carried out using a Hewlett-Packard 5989b quadrupole instrument (Vienna, Austria). Proton and Carbon NMR spectra were obtained on a Bruker AC 250 instrument (Follanden, Switzerland), operating at 250 MHz, calibrated with the solvent reference peak or TMS. IR spectra were plotted from KBr discs on a Mattson 300 FTIR Spectrometer. Melting points were recorded from a Stuart Scientific (Coventry, UK) Melting Point and are uncorrected.

1.1.1 Synthesis of 3,4-dichloro-5-phenyl-5H-furan-2-one, Lactone A

Dry and powdered aluminium chloride (20 g, 0.15 mol) was added slowly to a mixture of mucochloric acid (16.9 g, 0.1 mol) and benzene, fluorobenzene or chlorobenzene (250 ml). The reaction mixture was stirred overnight. For work up it was poured into a mixture of 100 g ice and 32 ml concentrated hydrochloric acid. The organic layer was separated by a separating funnel and it was washed with 3 x 100 ml of water. The combined organic layers were dried over magnesium sulphate and the solvent was removed under vacuum. The oily residue was crystallized in n-hexane.

Yield = 70%, M.P: 78-79^{o}C; R_f (80% ether / 20% petrol ether) = 0.62; Molecular Weight: 229.1; Molecular Formula: $C_{10}H_6Cl_2O_2$; MS (APCI(+)): 195/197 (M+), 230/232 (M+1) m/z; ^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.22-7.51 (m, ArH, 5H), 5.81 (CH) ppm. ^{13}C NMR (DCl_3) 250 MHz: δ = 165.3 (C=O), 152.2 (CH-C-Cl), 139.8 (ArC), 130.5, 129.3, 128.5, 127.4, 127.2 (5xArC), 121.2 (C=O-C-Cl), 83.5 (CH) ppm. IR (KBr-disc) υ_{max}: 3445, 3074, 3035, 2959, 2056, 1768, 1630, 1499, 1457 1294, 1224, 1028, 910, 772, 705 cm^{-1}.

1.1.2 3,4-Dichloro-5-(4-chloro-phenyl)-5H-furan-2-one, Lactone B

Yield = 69% M.P: 76-78^{o}C; R_f (80% ether / 20% petrol ether) = 0.55; Molecular Weight: 263.5; Molecular Formula: $C_{10}H_5Cl_3O_2$; MS (APCI(+)): 227/229/231 (M+1), 262/263/265 (M+) m/z; ^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.42-7.55 (m, ArH, 2H), 7.28-7.40 (m, ArH, 2H), 5.91 (CH) ppm.^{13}C NMR ($CDCl_3$) 250 MHz: δ = 165.3 (C=O), 152.0 (CH-C-Cl), 136.6 (ArC), 130.1 (ArC), 129.6 (2xArC), 128.7 (2xArC), 121.3 (CO-C-Cl), 82.9 (CH) ppm. IR (KBr-disc) υ_{max}: 3451, 3075, 2952, 2051, 1769, 1636, 1497, 1419, 1289, 1231, 1027, 927, 826, 748, 720 cm^{-1}.

1.1.3 3,4-Dichloro-5-(4-fluoro-phenyl)-5H-furan-2-one, Lactone C

Yield = 79% M.P: 74-76 ^{o}C; R_f (80% ether / 20% petrol ether) = 0.53; MS (APCI(+)): 227/229/231 (M+1), 247, 246 (M+) m/z; ^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.42-7.55 (m, ArH, 2H), 7.28-7.40 (m, ArH, 2H), 5.91 (CH) ppm. ^{13}C NMR ($CDCl_3$) 250 MHz: δ = 165.2 (C=O), 152.0 (CH-C-Cl), 136.6 (ArC), 130.1 (ArC), 129.6 (2xArC), 128.7 (2xArC), 121.3 (CO-C-Cl), 82.8 (CH) p.p.m. IR (KBr-disc) υ_{max}: 3451, 3075, 2952, 2051, 1769, 1636, 1497, 1419, 1289, 1231, 1027, 927, 826, 748, 720 cm^{-1}.

1.2 General Method

The relevant amine in 2.5 times excess was added to a solution of lactone **A, B**, or **C** (0.7 mol) in ether (10 ml) and the reaction mixture was stirred on ice for 30 minutes, allowing to warm up to RT over time. The resultant mixture was poured into 15 ml water and the organic phase was separated by a separating funnel. The mixture was subsequently washed with an excess of water for three more times. The organic layer was dried over magnesium sulphate and the solvent was removed under vacuum. All compounds gave an oily solid on a small scale, which was purified using column chromatography with a mixture of 80% ether / 20% petrol ether to yield crystals after removal of the solvent in vacuum.

1.2.1 4-Chloro-5-hydroxy-1-methyl-5-phenyl-1,5-dihydro-pyrrol-2-one 1

Yield = 75 %; Melting Point: 146-148 ^{o}C; R_f (80% ether / 20% petrol ether) = 0.26; Molecular Weight: 223.7; Molecular Formula: $C_{11}H_{10}ClNO_2$; MS (APCI(+)): 193/195 (M+1), 224/226 (M+) m/z; ^{1}H NMR (DMSO-d_6)) 250 MHz: δ = 7.29-7.48 (m, ArH, 5H), 6.49 (s, CH), 2.08 (s, CH_3) ppm. ^{13}C NMR ($CDCl_3$) 250 MHz: δ = 168.1 (C=O), 156.4 (C-Cl), 134.1 (ArC), 129.4 (2xArC), 128.9 (2xArC), 126.2 (ArC), 121.3 (CH-Cl), 92.6 (C-OH), 24.5 (CH_3) ppm. IR (KBr-disc) υ_{max}: 3224, 3110, 2952, 2820, 2617, 2375, 2339, 1975, 1697, 1605, 1453, 1438, 1258, 1207, 1065, 992, 856, 764, 704 cm^{-1}.

1.2.2 4-Chloro-5-(4-chloro-phenyl)-5-hydroxy-1-methyl-1,5-dihydro-pyrrol-2-one 2

Yield = 66 %; Melting Point: 179-181 ^{o}C; R_f (80% ether / 20% petrol ether) = 0.24; Molecular Weight: 258.1; Molecular Formula: $C_{11}H_9Cl_2NO_2$; MS (APCI(+)): 227/229/231 (M+1), 258/260/262 (M+) m/z; ^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.31-7.42 (ArH, 4H), 6.06 (s, CH), 4.56-4.71 (bs, OH), 2.60 (s, CH_3) ppm.^{13}C NMR ($CDCl_3$) 250 MHz: δ = 167.8 (C=O), 156.0 (C-Cl), 135.5 (ArC), 132.8 (ArC), 129.1 (2xArC), 127.8 (2xArC), 121.6 (CH-CCl), 92.2 (C-OH), 24.4 (CH_3) ppm. IR (KBr-disc) υ_{max}: 3429, 3102, 2970, 2932, 2857, 1677, 1611, 1494, 1475, 1431, 1202, 1151, 1091, 988, 928, 811, 692 cm^{-1}.

1.2.3 4-Chloro-5-hydroxy-1-isopropyl-5-phenyl-1,5-dihydro-pyrrol-2-one 3

Yield = 79 %; Melting Point: 163-165 ^{o}C; R_f (80% ether / 20% petrol ether) = 0.26; Molecular Weight: 251.7; Molecular Formula: $C_{13}H_{14}ClNO_{2;}$ MS (APCI(+)): 193/195 (M+1), 252/254 (M+) m/z; ^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.40-7.51 (m, ArH, 5H), 6.14 (s, CH), 3.71-3.79 (bs, OH), 3.42-3.59 (m, N-CH, *J* = 7.5 Hz), 1.33-1.48 & 1.21-1.28 (d, CH_3, 6H) ppm. ^{13}C NMR ($CDCl_3$) 250 MHz: δ = 167.5, 155.0, 135.0, (ArC), 129.1 (2xArC), 128.5 (2xArC), 126.4 (ArC), 122.4 (CH-CCl), 93.4 (C-OH), 45.6 (N-CH), 21.1, 20.0 (CH_3, 2xC) ppm. IR (KBr-disc) υ_{max}: 3227, 2990, 2940, 2365, 2350, 1956, 1693, 1615, 1456, 1428, 1247, 1131, 1072, 1009, 934, 847, 747, 697 cm^{-1}.

1.2.4 4-Chloro-5-(4-chloro-phenyl)-5-hydroxy-1-isopropyl-1,5-dihydro-pyrrol-2-one 4

Yield =69 %; Melting Point: 127-130 ^{o}C; R_f (80% ether / 20% petrol ether) = 0.21; Molecular Weight: 286.2; Molecular Formula: $C_{13}H_{13}Cl_2NO_{2;}$ MS (APCI(+)):227/229231 (M+1), 286/288/290 (M+) m/z; ^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.31-7.48 (m, ArH, 4H), 6.06 (s, CH), 3.33-3.52 (m, N-CH), 1.25-1.37 & 1.10-1.22 (d, CH_3, 6H), (OH not detected) ppm. ^{13}C NMR ($CDCl_3$) 250 MHz: δ = 167.1 (C=O), 154.0 (C-Cl), 136.7 (ArC), 133.4 (ArC), 128.9 (2xArC), 128.0 (2xArC), 123.2 (CH-CCl), 92.9 (C-OH), 45.6 (N-CH), 20.1 & 21.3 (CH_3, 2xC) ppm. IR (KBr-disc) υ_{max}: 3272, 2978, 2927, 1691, 1614, 1496, 1429, 1384, 1352, 1249, 1096, 1012, 936, 846, 801, 683 cm^{-1}.

1.2.5 4-Chloro-1-cyclopropyl-5-hydroxy-5-phenyl-1,5-dihydro-pyrrol-2-one 5

Yield = 83 %; Melting Point: 177-179 ^{o}C; R_f (80% ether / 20% petrol ether) = 0.24; Molecular Weight: 249.7; Molecular Formula: $C_{13}H_{12}ClNO_{2;}$ MS (APCI(+)): 193/195 (M+1), 250/252 (M+) m/z; ^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.31-7.49 (ArH, 5H), 6.09 (s, CH), 3.41-3.50 (C-OH), 2.08-2.21 (m, N-CH), 0.95-1.04 & 0.38-0.69 (m, CH_2, 4H) ppm. ^{13}C NMR ($CDCl_3$) 250 MHz: δ = 167.4 (C=O), 154.8 (C-Cl), 135.2 (ArC), 129.2 (2xArC), 128.8 (2xArC), 126.1 (ArC), 122.2 (CH-CCl), 93.5 (C-OH), 22.6 (N-CH), 3.8, 5.1 (CH_2, 2C) ppm. IR (KBr-disc) υ_{max}: 3416, 3260, 3105, 3011, 2363, 2338, 1671, 1602, 1490, 1450, 1409, 1369, 1256, 1144, 1032, 939, 833, 752, 702 cm^{-1}.

1.2.6 4-Chloro-5-(4-chloro-phenyl)-1-cyclopropyl-5-hydroxy-1,5-dihydro-pyrrol -2-one 6

Yield = 72 %; Melting Point: 169-171 ^{o}C; R_f (80% ether / 20% petrol ether) = 0.19; Molecular Weight: 284.1; Molecular Formula: $C_{13}H_{11}Cl_2NO_{2;}$ MS APCI(+)):227/229/231 (M+1), 284/286/288 (M+) m/z; ^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.12-7.32 (m, ArH, 4H), 5.97 (s, CH), 3.98-4.16 (bs, OH, 1.67-1.82 (m, N-CH), 0.24-0.99 (m, overlapping CH_2, 4H) ppm. ^{13}C NMR ($CDCl_3$) 250 MHz: δ = 165.8 (C=O), 155.4 (C-Cl), 144.2 (ArC), 133.7 (ArC), 129.0 (2xArC), 127.7 (2xArC), 122.2 (CH-CCl), 91.7 (C-OH), 22.6 (N-CH),

3.7 & 5.2 (CH_2, 2xC) ppm. IR (KBr-disc) υ_{max}: 3433, 3220, 3019, 2935, 2858, 1700, 1675, 1497, 1412, 1251, 1209, 1144, 1089, 1015, 940, 844, 802, 679 cm^{-1}.

1.2.7 4-Chloro-1-cyclopentyl-5-hydroxy-5-phenyl-1,5-dihydro-pyrrol-2-one 7

Yield = 81 %; Melting Point: 180-182 ^oC; R_f (80% ether / 20% petrol ether) = 0.26; Molecular Weight: 277.8; Molecular Formula: $C_{15}H_{16}ClNO_2$; MS (APCI(+)): 193/195 (M+1), 278/280 (M+) m/z; ^1H NMR ($CDCl_3$) 250 MHz: δ = 7.39-7.61 (m, ArH, 5H), 6.08 (s, CH), 4.77-4.92 (bs, OH), 3.49-3.68 (m, N-CH, *J* = 8.9 Hz), 1.98-2.17 (m, CH_2), 1.71-1.96 (m, CH_2, 4H), 1.36-1.55 (m, CH_2, 4H) ppm. ^{13}C NMR ($CDCl_3$) 250 MHz: δ = 167.2 (C=O), 155.0 (C-Cl), 135.2 (ArC), 129.1 (2xArC), 128.6 (2xArC), 126.5 (ArC), 122.2 (CH-CCl), 93.3 (C-OH), 54.3 (N-CH), 30.0 (CH_2), 28.8 (CH_2), 24.5, 24.4 (CH_2, 2xC) ppm. IR (KBr-disc) υ_{max}: 3220, 2961, 2877, 2373, 2341, 1684, 1613, 1448, 1426, 1248, 1199, 1141, 1070, 934, 850, 750, 701 cm^{-1}.

1.2.8 4-Chloro-5-(4-chloro-phenyl)-1-cyclopentyl-5-hydroxy-1,5-dihydro-pyrrol-2-one 8

Yield = 73 %; Melting Point: 157-159 ^oC; R_f (80% ether / 20% petrol ether) = 0.23; Molecular Weight: 312.2; Molecular Formula: $C_{15}H_{15}Cl_2NO_2$; MS (APCI(+)): 227/229/231 (M+1), 312/314/316 (M+) m/z; ^1H NMR ($CDCl_3$) 250 MHz: δ = 7.32-7.51 (ArH, 4H), 6.03 (s, CH), 4.95-5.03 (bs, OH), 3.41-3.62 (m, N-CH, *J* = 9.26 Hz), 1.97-2.19 (m, CH_2), 1.68-1.93 (m, overlapping CH_2, 8H) ppm. ^{13}C NMR ($CDCl_3$) 250 MHz: δ = 167.1 (C=O), 154.8 (CH-CCl), 135.2 (ArC), 133.9 (ArC), 128.9 (2xArC), 128.0 (2xArC), 122.3 (CH-CO), 93.0 (C-OH), 54.3 (N-CH), 30.0 & 28.9 (N-CH-CH_2, 4xC), 24.5 (N-CH-CH_2-CH_2, 2xC) ppm. IR (KBr-disc) υ_{max}: 3407, 3276, 2968, 2922, 2883, 2379, 2339, 1691, 1491, 1429, 1367, 1249, 1203, 1092, 1013, 932, 843, 787, 709 cm^{-1}.

1.2.9 4-Chloro-5-hydroxy-1-isobutyl-5-phenyl-1,5-dihydro-pyrrol-2-one 9

Yield = 85 %; Melting Point: 167-169 ^oC; R_f (80% ether / 20% petrol ether) = 0.27; Molecular Weight: 264.7;Molecular Formula: $C_{14}H_{16}ClNO_2$; MS (APCI(+)): 193/195 (M+1), 266/268 (M+) m/z; ^1H NMR ($CDCl_3$) 250 MHz: δ = 7.38-7.51 (m, ArH, 5H), 6.24 (s, CH), 4.79-4.98 (bs, OH), 3.23-3.32 & 2.18-2.29 (dd, CH_2, *J* = 8.1 Hz, 2H), 1.71-1.90 (m, CH-CH_2, *J* = 7.4) Hz), 0.76-0.96 (m, CH_3, 6H) ppm. ^{13}C NMR ($CDCl_3$) 250 MHz: δ = 168.5 (C=O), 155.7 (CH-CCl), 137.1 (ArC), 129.2, 128.7, 126.2 (5xArC), 121.7 (CH-CCl), 93.1 (C-OH), 47.6 (CH_2), 27.5 (CH-CH_2), 20.4 (CH_3, 2xC) ppm. IR (KBr-disc) υ_{max}: 3237, 3114, 2965, 2926, 2881, 2374, 2343, 1675, 1614, 1460, 1416, 1299, 1251, 1202, 1150, 1072, 1027, 878, 758, 696 cm^{-1}.

Crystal data - (sample recrystallised from methanol):

$C_{28}H_{32}Cl_2N_2O_4$	V = 1371.5(5) Å^3
M_r = 531.46	Z = 2
T = 293(2) K	D_x = 1.287 Mg/m^{-3}
Tabular	D_m not measured
0.20 x 0.15 x 0.10 mm	R [$F^2 > 2\sigma(F^2)$] = 0.0541
Colourless	wR(F^2) = 0.1165
Mo Kα radiation: λ = 0.71073 Å	5136 reflections
Triclinic	331 parameters
P-1	
a = 8.3190(13) Å	
b = 12.614(4) Å	
c = 13.8106(18) Å	
α = 93.049(17) °	
β = 94.791(12) °	
ϒ = 107.651(19) °	

Selected geometric parameters (Å, °)

Cl(1)-C(1)	1.696(4)	Cl(1')-C(1')	1.695(4)
C(1)-C(4)	1.310(5)	C(1')-C(4')	1.322(5)
C(2)-C(5)	1.511(5)	C(2')-C(5')	1.524(5)
C(2)-O(2)	1.410(4)	C(2')-O(2')	1.400(4)
C(3)-O(1)	1.224(5)	C(3')-O(1')	1.237(4)
N(1)-C(11)	1.448(5)	N(1)-C(11)	1.448(5)

1.2.10 4-Chloro-5-(4-fluoro-phenyl)-5-hydroxy-1-isobutyl-1,5-dihydro-pyrrol-2-one 10

Yield = 86%; Melting Point: 158-159 °C; R_f (80% ether / 20% petrol ether) = 0.21; Molecular Weight: 284.2; Molecular Formula: $C_{14}H_{15}ClFNO_2$; MS (APCI(+)): 211/212(M+1), 284/285 (M+) m/z; ^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.30-7.41 (m, ArH, 4H), 6.19 (s, CH), 3.13-3.31 (dd, CH_2, *J* = 8.0 Hz, 1H), 2.49-2.62 (dd, CH_2, *J* = 8.0 Hz, 1H), 1.69-1.83 (m, CH, *J* = 5.8 Hz), 0.69-0.80 (t, CH_3, *J* = 4.5 Hz, 6H) ppm.^{13}C NMR ($CDCl_3$) 250 MHz: δ = 163.6 (C=O), 156.1 (CH-<u>C</u>Cl), 139.0 (ArC), 134.7 (ArC), 129.0 (2xArC), 127.2 (2xArC), 122.1 (<u>C</u>H-CCl), 95.0 (C-OH), 47.1 (CH_2), 27.2 (<u>C</u>H-CH_2), 20.5 (CH_3, 2xC) ppm IR (KBr-disc) υ_{max}: 3425, 3251, 2964, 2924, 2850, 1685, 1406, 1209, 1095, 816, 743, 703 cm^{-1}.

1.2.11 4-Chloro-5-(4-chloro-phenyl)-5-hydroxy-1-isobutyl-1,5-dihydro-pyrrol-2-one 11

Yield = 76%; Melting Point: 155-158 °C; R_f (80% ether / 20% petrol ether) = 0.22; Molecular Weight: 300.2; Molecular Formula: $C_{14}H_{15}Cl_2NO_2$; MS (APCI(+)): 227/229/231 (M+1), 300/302/304 (M+) m/z; ^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.30-7.41 (m, ArH, 4H), 6.19 (s, CH), 3.13-3.31 (dd, CH_2, *J* = 8.0 Hz, 1H), 2.49-2.62 (dd, CH_2, *J* = 8.0 Hz, 1H), 1.69-1.83 (m, CH, *J* = 5.8 Hz), 0.69-0.80 (t, CH_3, *J* = 4.5 Hz, 6H) ppm. ^{13}C NMR ($CDCl_3$) 250 MHz: δ = 163.3 (C=O), 156.3 (CH-<u>C</u>Cl), 139.4 (ArC), 134.8 (ArC), 129.1

(2xArC), 127.7 (2xArC), 122.3 (CH-CCl), 95.0 (C-OH), 47.6 (CH_2), 27.6 (CH-CH_2), 20.4 (CH_3, 2xC) ppm IR (KBr-disc) υ_{max}: 3426, 3252, 2964, 2924, 2850, 1684, 1406, 1209, 1095, 817, 743, 703 cm^{-1}.

1.2.12 4-Chloro-1-hexyl-5-hydroxy-5-phenyl-1,5-dihydro-pyrrol-2-one 12

Yield = 51 %; Melting Point: 173-175 ^oC; R_f (80% ether / 20% petrol ether) = 0.28; Molecular Weight: 293.8; Molecular Formula: $C_{16}H_{20}ClNO_2$; MS (APCI(+)): 193/195 (M+1), 294/296 (M+) m/z; ^1H NMR ($CDCl_3$) 250 MHz: δ = 7.40-7.52 (m, ArH, 5H), 6.15 (s, CH), 4.76-4.82 (bs, OH), 3.28-3.49 (m, CH_2, 1H), 2.91-3.08 (m, CH_2, 1H), 1.09-1.59 (m, CH_2, overlapping, 8H), 0.78-0.92 (t, CH_3, *J* = 7.1 Hz) ppm. ^{13}C NMR ($CDCl_3$) 250 MHz: δ = 168.0 (C=O), 155.6 (C-Cl), 134.9 (ArC), 129.2 (2xArC), 128.7 (2xArC), 126.2 (ArC), 121.8 (CH-Cl), 93.0 (C-OH), 40.2, 31.3, 28.7, 26.8, 22.5 (CH_2, 5xC), 14.0 (CH_3) ppm. IR (KBr-disc) υ_{max}: 3245, 2930, 2865, 1689, 1658, 1494, 1453, 1412, 1365, 1321, 1150, 1069, 927, 753, 696 cm^{-1}.

1.2.13 4-Chloro-5-(4-chloro-phenyl)-1-hexyl-5-hydroxy-1,5-dihydro-pyrrol-2-one 13

Yield = 49 %; Melting Point: 169-172 ^oC; R_f (80% ether / 20% petrol ether) = 0.25; Molecular Weight: 328.2; Molecular Formula: $C_{16}H_{19}Cl_2NO_2$; MS (APCI(+)): 227/229/231 (M+1), 328/330/332 (M+) m/z; ^1H NMR ($CDCl_3$) 250 MHz: δ = 7.31-7.43 (m, ArH, 4H), 6.15 (s, CH), 3.24-3.44 (m, CH_2, 1H), 2.67-2.91 m, CH_2, 1H), 1.04-1.69 (m, overlapping CH_2, 8H), 0.74-0.89 (t, CH_3, *J* = 6.3 Hz) ppm. ^{13}C NMR ($CDCl_3$) 250 MHz: δ = 165.8 (C=O), 155.7 (C-Cl), 140.8 (ArC), 136.9 (ArC), 129.1 (2xArC), 127.8 (2xArC), 91.6 (C-OH), 40.3 (N-CH_2), 30.8 (N-CH_2-CH_2), 29.1 (N-CH_2-CH_2-CH_2), 26.8 (NH-CH_2-CH_2-CH_2-CH_2), 22.6 (CH_3-CH_2), 15.2 (CH_3) ppm. IR (KBr-disc) υ_{max}: 3446, 2935, 2863, 1698, 1413, 1252, 1200, 1138, 1092, 1013, 938, 846, 814, 702 cm^{-1}.

1.2.14 4-Chloro-1-cyclohexyl-5-hydroxy-5-phenyl-1,5-dihydro-pyrrol-2-one 14

Yield = 57 %; Melting Point: 170-172 ^oC; R_f (80% ether / 20% petrol ether) = 0.27; Molecular Weight: 291.8; Molecular Formula: $C_{16}H_{18}ClNO_2$; MS (APCI(+)): 193/195 (M+1), 292/294 (M+) m/z; ^1H NMR ($CDCl_3$) 250 MHz: δ = 7.26-7.61 (m, ArH, 5H), 6.08 (s, CH), 3.72-3.85 (bs, OH), 2.83-3.19 (m, N-CH), 1.21-2.07 (m, overlapping CH_2, 10H) ppm. ^{13}C NMR ($CDCl_3$) 250 MHz: δ = 163.9 (C=O), 153.9 (C-Cl), 135.0 (ArC), 129.25 (2xArC), 128.9 (2xArC), 126.4 (ArC), 122.9 (CH-CCl), 96.0 (C-OH), 53.6 (N-CH), 32.8 (CH_2), 31.1 (CH_2), 29.8 (CH_2), 26.2 (2xCH_2), 24.2 (CH_2) ppm. IR (KBr-disc) υ_{max}: 3440, 2924, 2858, 2355, 2344, 1641, 1449, 1367, 1250, 1138, 1016, 996,742, 695 cm^{-1}.

1.2.15 4-Chloro-1-(phenyl)-5-hydroxy-5-phenyl-1,5-dihydro-pyrrol-2-one 15

Yield = 39 %; M.P: 168-171 ^oC, R_f (80% ether / 20% petrol ether) = 0.19; Molecular Weight: 286.8; Molecular Formula: $C_{16}H_{12}ClNO_2$; MS (APCI(+)): 163/165 (M+1), 284/286 (M+) m/z; ^1H NMR ($CDCl_3$)

250 MHz: δ = 7.21-7.52 (m, Ar-H, 5H), 6.38 (s, CH-O), 3.68-3.73 (bs, C-OH) ppm. ^{13}C NMR ($CDCl_3$) 250 MHz: δ = 168.9 (C=O), 159.7 (C-Cl), 136.9 (ArC), 135.1 (ArC), 132.4 (ArC), 129.9 (ArC), 129.0 (2xArC), 126.9 (ArC), 126.1 (2xArC), 123.0 (ArC) 122.2 (CH-CCl), 93.5 (C-OH), ppm. IR (KBr-disc) υ_{max}: 3517, 3357, 3114, 2840, 2674, 2361, 2342, 1678, 1607, 1464, 1412, 1361, 1208, 1138, 1071, 988, 755, 700 cm^{-1}.

1.2.16 1-Benzyl-4-chloro-5-hydroxy-5-phenyl-1,5-dihydro-pyrrol-2-one 16

Yield = 71 %; Melting Point: 165-167 ^{o}C; R_f (80% ether / 20% petrol ether) = 0.21; Molecular Weight: 299.8; Molecular Formula: $C_{17}H_{14}ClNO_2$; MS (APCI(+)): 193/195 (M+1), 300/302 (M+) m/z; ^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.31-7.42 (m, ArH, 5H), 7.14-7.27 (m, ArH, 5H), 6.08 (s, CH), 4.59-4.70 (d, CH_2, 1H), 3.93-4.09 (d, CH_2, 1H), 3.52-3.79 (bs, OH) ppm. ^{13}C NMR ($CDCl_3$) 250 MHz: δ = 167.9 (C=O), 155.9 (C-Cl), 137.6 (ArC), 134.4 (ArC), 129.3 (ArC), 128.7 (4xArC), 128.4 (2xArC), 128.4 (ArC), 127.3 (ArC), 126.4 (ArC), 93.2 (C-OH), 43.4 (CH_2) ppm. IR (KBr-disc) υ_{max}: 3446, 3279, 3098, 2931, 2850, 2374, 2334, 1684, 1611, 1456, 1413, 1349, 1276, 1205, 1128, 1051, 696 cm^{-1}.

2. Pharmacology

2.1 $^{[125]}$I-CCK-8 Radioligand cholecystokinin binding assay

CCK_A and CCK_B receptor binding assays were performed, by using guinea pig cerebral cortex (CCK_2) or rat pancreas (CCK_1). Male guinea pig brain tissues were prepared according to the modified method described by Saita et al.[1] Pancreatic membranes were prepared as described by Charpentier et al.[2] Tissues were homogenized in ice cold sucrose (0.32 M, 25 ml) for 15 strokes at 500 rpm and centrifuged at 13000 rpm for 10 minutes. The supernatant was re-centrifuged at 13000 rpm for 20 minutes. The resulting pellet was re-dispersed to the required volume of buffer at 500 rpm and stored in aliquots at 70°C. Binding was achieved using radioligand ^{125}I-Bolton-Hunter labelled CCK, NEN at 25 pM. The samples were incubated with membranes (0.1 mg/ml) in 20 mM Hepes, 1mM EGTA, 5 mM $MgCl_2$, 150 mM NaCl, at pH 6.5 for 2 hrs at RT and then centrifuged at 11000 rpm for 5 minutes. The membrane pellets were washed twice with water and the bound radioactivity was measured in a Packard Cobra Auto-gamma counter (B5005). Binding assays were carried out with L-363, 260 as control.

2.2 Functional assays: Isolated tissue preparations

Adult male guinea pigs / rats, were used and all animal care and experimental protocols adhered to the relevant laws and guidelines of the institution. The animals were housed under standard conditions of temperature (25 ^{o}C) with unrestricted access to food and water. The animals were

sacrificed using cervical dislocation without anaesthesia. From the abdomen of the animals, the ileum was carefully excised at a site 15 cm away from the ileocaecal junction and washed with physiological solution. The mesentery of the ileum was removed and the ileal lumen was gently flushed with Tyrode's solution to clear luminal contents. The prepared isolated tissue was rapidly incubated in Tyrode's solution maintained at 32 ^{o}C and gassed with 95% O_2 / 5% CO_2.

Tyrode's solution was freshly prepared daily (g/l): NaCl, 8.0; KCl, 0.2; $CaCl_2$, 0.2; $MgSO_4$, 0.1; NaH_2PO_4, 0.05; $NaHCO_3$, 1.0; Glucose, 1.0.

The main equipment used was the Radnoti single unit tissue bath system with a chamber capacity of 35ml. Bath aeration with carbogen (O_2 95%, CO_2 5%) was maintained at a constant temperature (32°C). The force in grams was measured with an isometric transducer linked to a power lab data acquisition system.

General procedure

From the isolated tissue preparation, ileal strips of appropriate length were mounted vertically in organ bath containing Tyrode's solution, under a tension of 1 g and allowed to equilibrate for 30 minutes. Agonists were directly applied in the bath and antagonists were pre-incubated for about 10 min. Stock solutions of all test compounds used including the standard antagonist were prepared in DMSO.

Electrically stimulated muscle contractions

The intramural nerves within the ileal strips were excited by rectangular pulses of 2 ms, 25 mA and a frequency of 0.2 Hz. Transmural stimulation was applied using two platinum electrodes, one placed in the lumen of the ileum and the other outside the tissue.

CCK-8 stimulated isolated guinea pig gallbladder

CCK-8S was dissolved in distilled water to prepare a stock solution of 500 µM solution, from which cumulative additions of increasing concentrations (0.1 nM, 1 nM, 5 nM, 10 nM, 20 nM, 30 nM, and 40 nM) were tested to plot a dose response curve. Test molecules and Lorglumide were added to the organ bath 10 minutes before exposure to the next CCK-8S serial concentrations.

2.3 Anticancer assays

2.3.1 *In vitro cytotoxicty*

Cytotoxicity was determined against the murine and humane carcinoma cell lines, using the standard MTT assay.[3]

The culture media used was RPMI 1640 containing hepes, glutamine, antibiotics and supplemented with 10% fetal calf serum for MAC 13 cells and 5% fetal calf serum for MAC 16 cells. Cells were

counted by the trypan blue exclusion method using a plastic Kova counting chamber. MAC 16 cells were suspended in appropriate volumes of media and were seeded at 0.5 x 10^4 and 2 x 10^4 cells / 200 μl respectively in flat-bottomed 96 well plates. The test compounds were dissolved in dimethyl sulphoxide (DMSO) to give stock solutions of 100 mM. Dilution series from 10^{-4} M to 10^{-9} were made so that each compound was tested at six concentrations and in triplicate.

5-Fluoro-uridine (5-FU), a known anticancer agent, was used as a control and tested in the 20-0.02 μM range. Plates were then incubated at 37 °C, 5% CO_2 for three days. Compounds were tested on at least two separate occasions. On day three 20 μl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) (7.5mg MTT /ml of PBS) was added to each well and plates were allowed to incubate for a further 2 hrs. 120 μl of culture supernatant was carefully removed from each well and 100 μl of acidified i-propanol containing 10% Triton-X100 was then added to each well. Plates were agitated for 10 minutes at 800 rpm on a plate shaker. Following this solubilisation step all plates were then read, within 15 minutes, on an Anthos AW200 plate reader at 540 nm with a reference wavelength of 590 nm.

2.3.2 Xenograft study in NSG mice

1 million cells were used per mouse and the test molecule was administered in 20% DMS0 + 80% PEG-300. The suspension was vortexed and warmed at 37 °C for 5 min to ensure dissolution. MAC 16 mouse chemo resistant colon cancer cells were grown in RPMI medium supplemented with 10% fetal bovine serum (FBS) in 37 °C incubator with 5% CO_2. MIAPACA human pancreatic cancer cells were grown in DMEM supplemented with 10% FBS in 37 °C incubator with 5% CO_2. Cells were grown until 70% confluence before passaging into fresh flasks. For xenograft implantation, above indicated cells were harvested, viable cells determined by trypan blue exclusion, and a cell suspension in growth medium was prepared. The cell suspension in growth medium (100-111/mouse) was implanted subcutaneously in NSG mice. Once tumours reached 100 mm^3, the animals were randomized within the respective cell line and treated orally. Body weight and tumour volume were measured thrice weekly (Mon, Wed, and Fri). Animals were sacrificed (6 hrs after the last dose) when the tumours reached over 1500 mm^3 or when the animals lost over 20% body weight.

All experiments were performed in compliance with the relevant laws and institutional guidelines and the institutional bioethics committee has approved the experiments.

3. Molecular modeling

For target preparation the protein structures, having the pdb identifier 1HZN for the CCK_1 and 1L4T for the CCK_2 –gastrin receptor, were downloaded from the protein data bank (www.rcs.org) and docking was performed with Autodock Vina and Hex. After several docking trials for the CCK receptor the results were analysed and visualized using Chimera or Designer studio 4.5. After visual inspection and scores, results were presented to rationalize drug ligand interaction with the CCK receptor.

4. Statistical methods

The data were expressed as mean $\pm$ SD and one-way analysis of variance (ANOVA) and supplementary Tukey test for pairwise comparison were tested to determine for any significant difference at $p< 0.05$.

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References

1. Y. Saita, H. Yazawa, Y. Honma, A. Nishida, K. Miyata and K. Honda, *Eur. J. Pharmacol.*, 1994, **269**, 249.
2. B. Charpentier, D. Pelaprat, C. Durieux, A. Dor, M. Reibaud, J. C. Blanchard and B. P. Roques, *Proc. Natl. Acad. Sci. U S A*, 1988, **85**, 1968.
3. T. Mosmann, *J. Immunol. Methods,* 1983, **16**, 55.