Original Article

Cholecystokinin antagonists (part 1): Antinociceptive, anxiolytic and antidepressant effects of *N*-(5-methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-*N*'-phenylureas and carboxamides

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ABSTRACT: The SAR optimization of the pyrazoline template resulted in novel 3-oxo-1,2-diphenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-indole carboxamides and novel 3-oxo-1,2-diphenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-*N*'-phenylureas. These non-peptidal heterocyclic compounds have shown to bind as potent CCK_1 selective and mixed CCK antagonists in a [^{125}I]CCK-8 receptor binding assay. The best amides 3c and 3d of this series displayed an IC_{50} of 20 and 25 nM for the CCK_1 receptor, respectively. The best ureido-pyrazoline 4b and 4e of this series displayed an IC_{50} of 20 and 25 nM, as a mixed CCK receptor antagonist. In the elevated x-maze an anxiolytic effect of the urea 4e was found from 10 µg/kg upwards for the mixed antagonist. In the despair swimming test, a model for testing antidepressants, both mixed and CCK_1 selective antagonists were found active as a modulator over a big range from from 10-500 µg/kg and the magnitude of the effects were comparable to desimipramine. The amides and the phenylureas enhanced significantly the analgesic effect of morphine over a wide dose range in mice.

Keywords: CCK-antagonists, *N*-(3-Oxo-2,3-dihydro-1*H*-pyrazol-4-yl)-indole-carboxamides, 3-Oxo-1,2-diphenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-*N*'-phenyl ureas, Elevated plus-maze, Forced swim test, Tail immersion test

1. Introduction

Cholecystokinin, which act as a neuromodulator/gut hormone and CCK-ligands, agonists as well as antagonists (*1*), have been extensively investigated as potential drug targets (*2*). CCK-antagonists were studied as growth inhibitors in certain forms of cancer (*3*), as anxiolytics (*4*), in the treatment of schizophrenia (*5*), satiety (*6*) and as anti-panic agents (*7*). An agonist, the shortened CCK tetrapeptide, was found to induce panic in patients (*8*). A phase II trial of Devazepide, a potent and CCK_1 selective antagonist (*9*), has been recently completed (*10*) showing a significant enhancement of the effect of morphine in the treatment of chronic and severe pain (*11*).

Asperlicin, a microbial metabolite, was the first non-peptidal cholecystokinin antagonist and analogues thereof, were studies as CCK ligands (*12*). Simplification of the lead structure from nature led to Devazepide, a potent CCK_1 selective cholecystokinin antagonist, containing a 1,4-benzodiazepine template and an indole moiety. The 1,4-benzodiazepine template was varied by a combinatorial solid phase synthesis (*13*) and was optimized in terms of CCK binding affinity (*14*).

In the search for new CCK ligands, in which the 1,4-benzodiazepine structure was replaced by an achiral template, the diphenyl pyrazolone template was selected as starting point.

The combination of indole carboxylic acids (*15*) and phenyl ureas (*16*) with amino-pyrazolines resulted in the discovery of potent lead structures and the results are reported in this publication.

Traditionally, the pyrazoline template had been used for anti-pyretic, anti-rheumatic and analgesic drugs (*17*). Having realized the relevance of the CCK_1 receptor in the treatment of pain (*18*) and depression (*19*), indole amides and phenyl ureas of the pyrazoline template were prepared by a short synthetic approach and evaluated in receptor binding assays. The aim of our study was to convert a template, usually linked with non-steroidal anti-inflammatory agents into a CNS drug, thus creating a non-benzodiazepine template (*20*)

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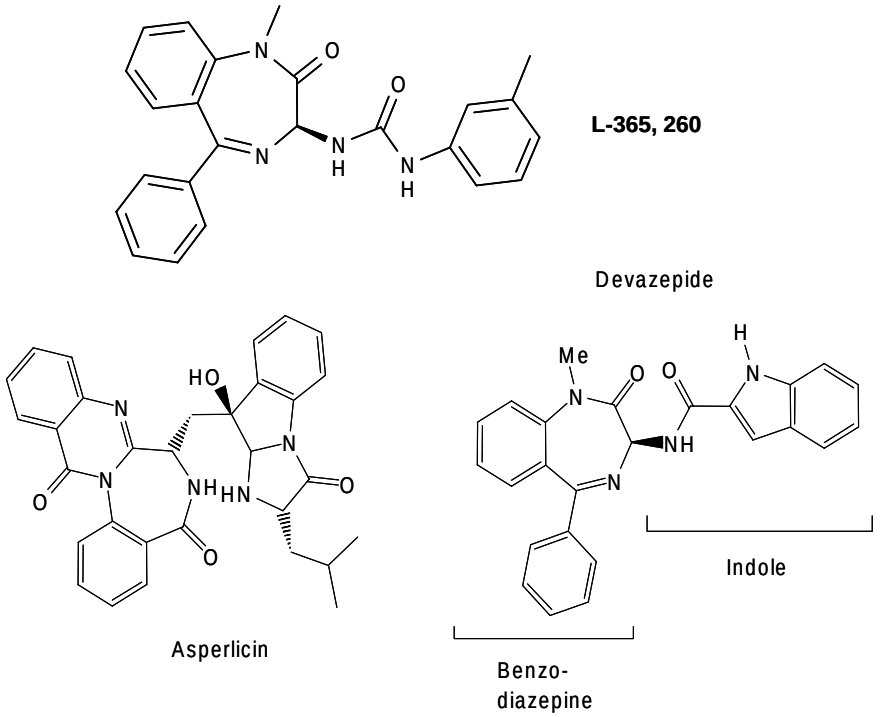


Figure 1. Development of cholecystokinin ligands.

based cholecystokinin antagonist.

The synthesis of novel diphenyl pyrazolinyl amides and ureas, the receptor binding properties on the CCK receptor subtypes and the evaluation of the most potent ligands in various animal models is reported. It is supposed that the 1,5-diphenylpyrazoline moiety is able to mimic the receptor interactions of the 1,4-benzodiazepine scaffold.

2. Materials and Methods

2.1. *Synthesis*

The chemicals were obtained from Aldrich (Gillingham, UK) and Lancaster (Lancaster, UK). Atmospheric pressure chemical ionization 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.

2.1.1. *Synthesis of 5-methyl-1,2-diphenyl-1,2-dihydro-3H-pyrazol-3-one* ***1***

Diphenyl hydrazine (50.0 g, 0.27 mol) and aceto ethyl acetate (2 Eq. 69.0 mL, 0.52 mol) were heated at 130-150°C for 2 h, with a Dean stark trap. The mixture was then heated for an additional 1.5 h to 180°C, to remove water and ethanol. The remaining solution was distilled at 230-250°C/2mm Hg. This removed any unreacted diphenyl hydrazine to give a viscous black liquid. The mixture was allowed to cool to RT and then ether was added to precipitate out crude black crystals. These were subsequently recrystallized twice from toluene. Yield: 22.1 g, 32.8%. Mol. Weight: 250.3. Mol. Formula: $C_{16}H_{14}N_2O$. MS (APCI(+)): 251 (M+1) m/z. IR (KBr disc): 3465, 3090, 1671, 1590, 1490, 1380, 1349, 1241, 971, 753 and 688 cm^{-1}. ^1H NMR ($CDCl_3$) 300K: 2.07 (s, CH_3), 5.55 (s, CH), 7.05-7.37 (m, Ar-10H) p.p.m. ^{13}C NMR ($CDCl_3$) 300K: 13.7 (CH_3), 99.2 (CH), 123.6, 125.5, 125.9, 128.0, 128.6, 129.3, 135.7, 139.0, 156.3 (C-N), 166.5 (C=O) p.p.m.

2.1.2. *Synthesis of 4-amino-5-methyl-1,2-diphenyl-1,2-dihydro-3H-pyrazol-3-one* ***2*** *via the synthesis of 4-nitroso-5-methyl-1,2-diphenyl-1,2-dihydro-3H-pyrazol-3-one*

5-Methyl-1,2-diphenyl-1,2-dihydro-3*H*-pyrazol-3-one (10.0 g, 0.04 mol) was warmed in HCl (conc) (60.0 mL), when dissolved the solution was diluted with water (up to 400 mL). Sodium nitrite (2.8 g, 0.041 mol) in water (50.0 mL) was added in drops to the mixture at 0°C, whilst stirring. A green precipitate was produced, which was allowed to stand for 45 min, then was filtered, washed with cold water and was reacted further into the amine **2**.

The 4-nitroso-5-methyl-1,2-diphenyl-1,2-dihydro-3*H*-pyrazol-3-one intermediate was dissolved in ethanol (250 mL). A mixture of tin chloride (20.4 g, 0.11 mol) in 20% HCl (120 mL) was heated to 90°C. When

dissolved, this hot mixture was added to the alcoholic solution of the nitroso-intermediate and the mixture was allowed to cool to RT overnight. Ammonia solution (conc 33%) was added to the mixture until no further precipitation occurred. The mixture was filtered, dried and extracted several times with ethanol. The ethanol was removed in *vacuo* and the crude mixture was recrystallized in ethanol to give bright yellow crystals.

Yield: 3.9 g, 37.0%. Mol. Weight: 265.3. Mol. Formula: $C_{16}H_{15}N_3O$. MS (APCI(+)): 266 (M+1), 251 (M+) m/z. IR (KBr-disc): 3407, 3210, 1654, 1592, 1492, 1351, 1262, 751 and 690 cm^{-1}. ^1H NMR (DMSO-d_6) 300K: 1.88 (s, CH_3), 5.57 (s, NH), 7.05-7.12 (tt, Ar-H, J = 7.3 Hz), 7.20-7.45 (m, Ar-9H) p.p.m. ^{13}C NMR (DMSO-d_6) 300K: 11.09 (CH_3), 120.3, 122.5, 123.8, 125.5, 128.0, 129.1, 129.8, 136.4, 142.7, 156.3, 166.3 (C=O) p.p.m.

2.1.3. *Synthesis of N-(5-methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)-1H-indole-carboxamides* ***3a-3e***

General method: A solution of 4-amino-5-methyl-1,2-diphenyl-1,2-dihydro-3*H*-pyrazol-3-one (0.2 g, 0.76 mmols) was dissolved in dry acetonitrile (20 mL). The appropriate indole acid (1.25 eq) was added, with DIC (3 eq). The mixture was heated to 60°C and left overnight. The resulting precipitated crystals were filtered, washed and dried.

N-(5-Methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)-1H-indole-2-carboxamide ***3a***

Yield: 201 mg, 65%. Mol. Weight: 408.5. Mol. Formula: $C_{22}H_{20}N_4O_2$. MS (APCI(-)): 409 (M+1), 408 (M+), m/z. IR (KBr-disc): 3401, 3339, 2965, 2358, 1710, 1615, 1583, 1454, 1361, 1172 and 748 cm^{-1}.

N-(5-Methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)-1H-indole-3-carboxamide ***3b***

Yield: 241 mg, 78%. Mol. Weight: 408.5. Mol. Formula: $C_{25}H_{20}N_4O_2$. MS (APCI(+)): 409 (M+1) m/z. IR (KBr-disc): 3343, 2965, 1615, 1581, 1535, 1494, 1453, 1318, 1249, 1191 and 750 cm^{-1}. ^1H NMR (DMSO-d_6) 300K: 2.04 (s, CH_3), 7.09-7.20 (m, Ar-3H), 7.27-7.45 (m, Ar-10H), 7.44-7.47 (d, Ar-H, J = 7.0 Hz), 7.99 (s, Ar-H), 9.16 (s, NH), 11.69 (s, NH) p.p.m. ^{13}C NMR (DMSO-d_6) 300K: 12.6 (CH_3), 109.7 (C-NH), 112.4, 121.0, 121.1, 121.5, 122.6, 123.6, 126.1, 126.3, 126.9, 128.6, 129.2, 129.3, 130.1, 132.7, 136.4, 139.9, 152.8, 164.5, 171.9 (C=O) p.p.m.

N-(5-Methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)-2-(1H-indol-3-yl)acetamide ***3c***

Yield: 211 mg, 66%. Mol. Weight: 422.5. MS (APCI(+)): 423 (M+1) m/z. IR (KBr-disc) : 3337, 2965, 1679, 1648, 1629, 1592, 1525, 1488, 1312, 1243 and 749 cm^{-1}. ^1H NMR (DMSO-d_6) 300K: 1.86 (s, CH_3), 3.73 (s, CH_2), 6.94-7.00 (t, Ar-H, J = 8.0, 7.9 Hz), 7.03-7.09 (t, Ar-H, J = 8.2, 8.1 Hz), 7.10-7.17 (t, Ar-H, J = 6.8, 6.7 Hz), 7.27-7.38 (m, Ar-10H), 7.61-7.64 (d, Ar-H, J = 7.7 Hz), 9.38 (s, NH), 10.88 (s, NH) p.p.m. ^{13}C NMR (DMSO-d_6) 300K: 12.5 (CH_3), 23.8 (CH_2), 109.1 (C-NH), 109.4, 111.8, 118.4, 119.2, 121.5, 123.7, 124.4, 126.1, 126.4, 127.7, 128.6, 129.3, 130.0, 136.1, 136.6, 139.7, 151.9, 162.7, 170.8 (C=O) p.p.m. Anal. cal. for $C_{26}H_{22}N_4O_2$: C, 73.92; H, 5.25; N, 13.26; O, 7.57. Found C, 73.90; H, 5.22; N, 13.27; O, 7.61.

N-(5-Methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)-3-(1H-indol-3-yl)propanamide ***3d***

Yield: 261 mg, 79%. Mol. Weight: 436.5. MS (APCI(+)): 375 (M+1) m/z. IR (KBr-disc): 3436, 3284, 1640, 1590, 1548, 1490, 1459, 1317 and 753 cm^{-1}. ^1H NMR (DMSO-d_6) 300K: 1.84 (s, CH_3), 2.65-2.71 (t, CH_2, J = 7.2, 7.1 Hz), 2.98-3.04 (t, CH_2, J = 7.3, 7.4 Hz), 6.94-7.00 (t, Ar-H, J = 6.8, 6.8 Hz), 7.03-7.09 (t, Ar-H, J = 6.9, 6.9 Hz), 7.11-7.17 (m, Ar-2H), 7.27-7.41 (m, Ar-11H), 7.55-7.58 (d, Ar-H, J = 7.7 Hz), 9.27 (s, NH), 10.77 (s, NH) p.p.m. ^{13}C NMR (DMSO-d_6) 300K: 12.5 (CH_3), 21.4, 23.8 (CH_2), 109.3 (C-NH), 111.8, 114.1, 118.6, 118.8, 121.4, 122.8, 123.7, 126.1, 126.4, 127.6, 128.6, 129.3, 130.1, 136.2, 136.7, 139.9, 151.9, 162.7, 172.1 (C=O) p.p.m. Anal. cal. for $C_{27}H_{24}N_4O_2$: C, 74.29; H, 5.54; N, 12.84; O, 7.33. Found: C, 74.30; H, 5.53; N, 12.82; O, 7.35.

N-(5-Methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)-4-(1H-indol-3-yl)butanamide ***3e***

Yield: 273 mg, 80%. Mol. Weight: 450.5. Mol. Formula: $C_{28}H_{26}N_4O_2$. MS (APCI(+)): 450 (M+1) m/z. IR (KBr-disc): 3235, 3046, 1656, 1635, 1590, 1544, 1494, 1432, 1276 and 699 cm^{-1}. ^1H NMR (DMSO-d_6) 300K: 1.92-1.98 (m, CH_3, CH_2 (overlapping), 2.35-2.40 (t, CH_2, J = 7.3, 7.3 Hz), 2.71-2.77 (t, CH_2, J = 7.4, 7.5 Hz), 6.93-6.99 (t, Ar-H, J = 6.9, 7.2 Hz), 7.02-7.10 (t, Ar-H, J = 6.9, 6.9 Hz), 7.13-7.16 (t, Ar-2H, J = 7.3, 7.1 Hz), 7.24-7.42 (m, Ar-11H), 7.51-7.54 (d, Ar-H, J = 7.7 Hz), 9.20 (s, NH), 10.75 (s, NH) p.p.m. ^{13}C NMR (DMSO-d_6) 300K: 12.5 (CH_3), 23.8, 24.8, 26.7 (CH_2), 109.4 (C-NH), 111.8, 114.6, 118.4, 118.8, 121.3, 122.8, 123.7, 126.1, 126.4, 127.7, 128.6, 129.5, 130.1, 136.2, 136.8, 139.8, 152.0, 162.8, 172.4 (C=O) p.p.m.

2.1.4. *General experimental for the formation of diphenylpyrazolinylureas* ***4a-4o***

4-Amino-5-methyl-1,2-diphenyl-1,2-dihydro-3*H*-pyrazol-3-one **3** (0.1 g, 3.8×10^{-4} mol) in dry acetonitrile (10-15 mL) was stirred at room temperature. The

appropriate substituted isocyanate (1.3 eq) in dry acetonitrile was added slowly over 5 min, allowed to stir at room temperature or heated to 60°C and was left overnight. The precipitate that formed was filtered, washed (twice) with cold acetonitril and dried, to give the corresponding urea as a pure product.

N-(5-Methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)N'-3-methoxy-phenylurea ***4b***

Yield: 36%. Mol. Weight: 414.6. Mol. Formula: $C_{24}H_{22}N_4O_3$. MS (APCI(+)): 415 (M+1), 266 (M+) m/z. IR (KBr-disc): 3207, 1708, 1646, 1619, 1594, 1540, 1488, 1453, 1282, 761 and 697 cm^{-1}. ^1H NMR (DMSO-d_6) 300K: 2.02 (s, C-CH_3), 3.72 (s, OCH_3), 6.50-6.55 (dd, Ar-H, J = 8.2 Hz), 6.88-6.92 (Ar-H, J = 8.1 Hz), 7.12-7.18 (m, Ar-3H), 7.26-7.44 (m, Ar-9H), 7.57 (s, NH), 8.88 (s, NH) p.p.m. ^{13}C NMR (DMSO-d_6) 300K: 12.6 (C-CH_3), 55.4 (OCH_3), 99.7 (C-CH_3), 104.2, 107.7, 109.7, 110.8, 123.6 (2×C), 125.6 (2×C), 126.2, 128.6, (2×C), 130.0 (2×C), 136.1, 139.2, 141.6, 143.0, 151.2, 153.8 (Ar-C), 160.2, 163.0 (C=O) p.p.m.

1-(4-Methoxy-phenyl)-3-(5-methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)-urea ***4c***

Yield: 75%, Melting Point: 244-246°C, Rf (ether) = 0.14, Mol. Weight: 414.6. Mol. Formula: $C_{24}H_{22}N_4O_3$ MS (APCI(+)): 266 (M+), 415 (M+1) m/z, IR (KBr-disc): 3300, 3261, 2929, 1708, 1642, 1618, 1552, 1512, 1420, 1250, 1207, 1018, 833, 763, 697 cm^{-1}, ^1H NMR (DMSO-d_6) 250 MHz: 8.70 (s, NH), 7.57 (s, NH), 7.24-7.49 (m, 11H, Ar-H), 7.11-7.20 (m, Ar-H), 6.79-6.90 (d, Ar-H), 3.72 (s, CH_3-O), 2.02 (s, CH_3-C) p.p.m. ^{13}C NMR (DMSO-d_6) 250 MHz: 162.5 (C-C=O), 150.6 (NH-C=O), 154.3 (ArC), 153.6 (2×ArC), 139.4, 135.6 (2×ArC), 132.9 (C-CH_3), 129.5 (2×ArC), 128.7, 128.6 (2×ArC), 128.1 (2×ArC), 125.8 (2×ArC), 123.1 (2×ArC), 119.8 (2×ArC), 113.9 (ArC), 109.5 (C-C=O), 55.1 (CH_3-O), 12.1 (CH_3-C) p.p.m.

N-(5-Methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)N'-3-methylphenylurea ***4e***

Yield: 91%. Mol. Weight: 398.5. Mol. Formula: $C_{24}H_{22}N_4O_2$. MS (APCI(+)): 399 (M+1), 266 (M+) m/z. IR (KBr-disc): 3322, 1698, 1644, 1625, 1538, 1490, 1285, 1211, 759 and 697 cm^{-1}. ^1H NMR (DMSO-d_6) 300K: 2.01 (s, CH_3), 2.25 (s, C-CH_3), 6.75-7.78 (d, Ar-H, J = 7.2 Hz), 7.10-7.44 (m, Ar-13H), 7.59 (s, NH), 8.80 (NH) p.p.m. ^{13}C NMR (DMSO-d_6) 300K: 12.7 (C-CH_3), 21.7 (CH_3), 109.9, 115.7, 119.1, 123.0, 123.7 (2×C), 126.4, 128.7, 129.1 (2×C), 130.1 (2×C), 136.1, 138.4, 139.9, 140.2, 142.9, 151.1 (Ar-C), 153.9, 163.0 (C=O) p.p.m.

1-(5-Methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)-p-tolyl-urea ***4f***

Yield: 73%, Melting Point: 250-252°C, Rf (ether) = 0.15, Mol. Weight: 414.6. Mol. Formula: $C_{24}H_{22}N_4O_2$ MS (APCI(+)): 266 (M+), 399 (M+1) m/z, IR (KBr-disc): 3299, 3058, 2924, 2857, 2358, 2334, 1711, 1648, 1624, 1601, 1540, 1506, 1416, 1292, 1202, 756, 693 cm^{-1}. ^1H NMR (DMSO-d_6) 250 MHz: 8.80 (s, NH), 7.57 (s, NH), 7.24-7.49 (m, 11H, Ar-H), 7.06-7.28 (m, Ar-H), 2.27 (s, Ar-CH_3), 2.02 (s, CH_3-C) p.p.m. ^{13}C NMR (DMSO-d_6) 250 MHz: 162.6 (C-C=O), 150.6 (NH-C=O), 153.5 (2×ArC), 139.4, 137.2, 135.5 (3×ArC), 130.5 (C-CH_3), 129.5 (2×ArC), 129.1 (2×ArC), 128.7 (ArC), 128.1 (2×ArC), 125.7 (2×ArC), 123.1 (2×ArC), 118.1 (2×ArC), 109.4 (C-C=O), 20.3 (Ar-CH_3), 12.1 (CH_3-C) p.p.m.

N-(2-Chlorophenyl)-N'-(5-methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)urea ***4g***

Yield: 73%. Mol. Weight: 418.9. Mol. Formula: $C_{23}H_{19}N_4O_2$. MS (APCI(+)): 418, 420 (M+1), 266 (M+) m/z. IR (KBr-disc): 3293, 3212, 1710, 1621, 1590, 1530, 1488, 1422, 1291, 1191, 761 and 680 cm^{-1}. ^1H NMR (DMSO-d_6) 300K: 2.01 (s, CH_3), 6.97-7.03 (tt, Ar-H, J = 6.8 Hz), 7.11-7.18 (tt, Ar-H, J = 6.9, 6.8 Hz), 7.22-7.44 (m, Ar-12H), 7.89 (s, NH), 9.09 (s, NH) p.p.m. ^{13}C NMR (DMSO-d_6) 300K: 12.6 (CH_3), 109.5, 117.4, 118.3, 122.2, 123.7 (2×C), 126.2 (2×C), 128.7, 129.3 (2×C), 130.8 (2×C), 130.9, 133.7, 139.8, 141.5, 141.9, 151.4 (Ar-C), 153.8, 162.9 (C=O) p.p.m.

N-(4-Bromophenyl)-N'-(5-methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)urea ***4i***

Yield: 92%. Mol. Weight: 463.3. Mol. Formula: $C_{23}H_{19}BrN_4O_2$. MS (APCI(+)): 464, 466 (M+1), 266 (M+) m/z. IR (KBr-disc): 3285, 3062, 1704, 1644, 1490, 1534, 1486, 1288, 1209, 757 and 705 cm^{-1}. ^1H NMR (DMSO-d_6) 300K: 2.01 (s, CH_3), 6.50-6.52 (d, Ar-H, J = 6.9 Hz), 7.01-7.17 (m, Ar-2H), 7.29-7.43 (m, Ar-11H), 7.65 (s, NH), 9.02 (s, NH) p.p.m. ^{13}C NMR (DMSO-d_6) 300K: 12.6 (CH_3), 109.6, 113.9, 116.3, 120.7, 123.7 (2×C), 125.9 (2×C), 126.2, 128.7 (2×C), 129.3 (2×C), 130.5, 131.9, 132.0 (2×C), 136.1 (2×C), 139.8, 153.8 (Ar-C), 157.8, 162.9 (C=O) p.p.m.

N-(5-Methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)N'-2-nitrophenylurea ***4j***

Yield: 65%. Mol. Weight: 430.4. Mol. Formula: $C_{23}H_{20}N_5O_4$. MS (APCI(+)): 431 (M+1), 266 (M+) m/z. IR (KBr-disc): 3318, 3181, 3010, 1712, 1658, 1635, 1588, 1502, 1432, 1344, 1272, 1201, 759 and 688 cm^{-1}. ^1H NMR (DMSO-d_6) 300K: 2.02 (s, CH_3), 7.12-7.44 (m, Ar-11H), 7.64-7.71 (t, Ar-H, J = 7.3, 7.4 Hz), 8.06-8.10 (d, Ar-H, J = 8.4 Hz), 8.28-8.32 (d, Ar-H, J = 8.5 Hz),

8.90 (s, NH), 9.71 (s, NH) p.p.m. ^{13}C NMR (DMSO-d_6) 300K: 12.4 (CH_3), 122.7, 123.9, 124.0, 125.9, 126.3 (2×C), 126.6, 127.3 (2×C), 128.8, 128.9, 129.3 (2×C), 130.1 (2×C), 133.2, 135.6, 136.0, 138.0, 139.6 (Ar-C), 153.5, 162.8 (C=O) p.p.m.

N-(5-Methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)-N'-phenylurea ***4l***

Yield: 91%. Mol. Weight: 384.4. Mol. Formula: $C_{23}H_{20}N_4O_4$. MS (APCI(+)): 385 (M+1), 266 (M+) m/z. IR (KBr-disc): 3420, 3297, 3072, 3065, 1706, 1640, 1544, 1492, 1448, 1297, 1202, 755 and 697 cm^{-1}. ^{1}H NMR (DMSO-d_6) 300K: 2.02 (s, CH_3), 6.91-6.97 (tt, Ar-H, J = 7.3 Hz), 7.11-7.17 (tt, Ar-H, J = 7.0, 7.1 Hz), 7.22-7.45 (m, Ar-13H), 7.60 (s, NH), 8.87 (s, NH) p.p.m. ^{13}C NMR (DMSO-d_6) 300K: 12.6 (CH_3), 109.8 (C-CH_3), 118.5 (2×C), 122.2, 122.4 (2×C), 123.7 (2×C), 125.9 (2×C), 126.2, 128.6 129.1 (2×C), 129.8 (2×C), 136.1, 139.9, 140.3, 151.1 (Ar-C), 153.8, 163.0 (C=O) p.p.m.

N-(5-Methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1H-pyrazol-4-yl)-N'-cyclohexylurea ***4n***

Yield: 86%. Mol. Weight: 390.5. Mol. Formula: $C_{23}H_{23}N_4O_2$. MS (APCI(+)): 391 (M+1), 266 (M+) m/z. IR (KBr-disc): 3359, 3299, 2929, 2849, 1636,1694, 1596, 1538, 1488, 1276, 1228, 763 cm^{-1}. ^{1}H NMR (DMSO-d_6) 300K: 1.10-1.88 (m, -CH, -CH_2-, 11H), 1.95 (s, CH_3), 6.27-6.30 (d, Ar-H, J = 7.9 Hz), 7.12-7.16 (tt, Ar-H, J = 6.8, 6.9 Hz), 7.24-7.42 (m, Ar-8H), 7.63 (s, NH), 8.86 (NH) p.p.m. ^{13}C NMR (DMSO-d_6) 300K: 12.9 (CH_3), 24.9 (-CH_2-×2), 25.8 (-CH_2-), 33.5 (-CH_2-×2), 48.5 (-CH-NH), 99.7 (C-CH_3), 110.0 (C-N), 123.5 (2×C), 126.1 (2×C), 128.5, 129.2 (2×C), 130.0 (2×C), 136.1, 140.3, 150.2 (Ar-C), 155.6, 163.1 (C=O) p.p.m.

2.2. *Pharmacology*

2.2.1. *Cholecystokinin binding assay, [^{125}I]CCK-8 receptor binding assay*

CCKA and CCKB receptor binding assays were performed, by using guinea pig cerebral cortex (CCKB) or rat pancreas (CCKA). Male guinea pig brain tissues were prepared according to the modified method described by Saita *et al.* (*21*). Pancreatic membranes were prepared as described by Charpentier *et al.* (*22*).

Tissues were homogenized in ice cold sucrose (0.32 M, 25 mL) for 15 strokes at 500 rpm and centrifuged at 13,000 rpm for 10 min. The supernatant was re-centrifuged at 13,000 rpm for 20 min. 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 labeled CCK, NEN at 25 pM. The samples were incubated with membranes (0.1 mg/mL) in 20 mM Hepes, 1 mM EGTA, 5 mM $MgCl_2$, 150 mM NaCl, at pH 6.5 for 2 h at RT and then centrifuged at 11,000 rpm for 5 min. The membrane pellets were washed twice with water and the bound radioactivity was measured in a Packard Cobra Auto-gamma counter (B5005). All binding assays were carried out with L-363, 260 as control.

2.2.2. *Animal studies*

Experiments were conducted in male standard IRC mice obtained from the animal house, Faculty of Medicine, Khon Kaen University. Each experimental group consisted of 6 animals and the treatment procedures were approved by the ethical committee, Faculty of Medicine, Khon Kaen University (BEA030699).

Mice were intraperitoneal injected with either test compound dissolved in 5% DMSO at the volume not more than 0.2 mL/animal. At 30 min after treatment, animals were tested as described in the following sections.

Anxiolytic activity tests, nociception tests and antidepression tests were performed as described in DDT 2007 (*19*).

2.2.3. *Anxiolytic activity tests*

The light/dark box: Mice were placed in the light part of the light/dark box. The box was a Plexiglass cage, 25×50×20 cm, having one-third as a dark and two-third as a light compartment. A 40-W light bulb was used and positioned 10 cm above the center of the light component. The animals could walk freely between dark and light parts through the opening. The time animals spent in light part during the 5 min interval was recorded. The mouse was considered to be in the light part when its 4 legs were in the light part.

The elevated plus-maze: The wooden elevated plus-maze consisted of two open arms (30×10 cm) without any walls, two enclosed arms of the same size with 5-cm high side walls and end wall, and the central arena (10 ×10 cm) interconnecting all the arms. The maze was elevated approximately 30 cm height from the floor. At the beginning of the experiment the mouse was placed in the central arena facing one of the enclosed arms. During a 5 min interval, the time animals spent in the open arms of plus-maze was recorded. The mouse was considered to be in the open part when it had clearly crossed the line between the central arena and the open arm with its 4 legs.

2.2.4. *Nociception tests*

The tail immersion test: The thermal response latency was measured by the tail immersion test. The animals were placed into individual restraining cages leaving

the tail hanging freely. The tail was immersed into water preset at 50°C. The response time, at which the animal reacted by withdrawing its tail from water, was recorded and the cut-off time was 10 sec in order to avoid damaging the animal's tissue.

The thermal response latency was measured by the tail immersion test. The base line withdrawal thresholds (BT) were recorded prior to the first injection. Test thresholds (TT) were measured 60 min after the second injection. The cut off time was set to 45 sec. This was to avoid any tissue damage to the paw during the course of analgesia testing. The test thresholds were expressed as a percentage of Maximal Possible Effect (% MPE) using the equation:

$$\% \text{ MPE} = \{(\text{TT-BT}) / (45\text{-BT})\} \times 100$$

The hot plate test: Mice were placed on a hot plate that was thermostatically maintained at 50°C. A Plexiglass box was used to confine the animal to the hot plate. The reaction time of each animal (either paw licking or jumping) was considered a pain response. The latency to reaction was recorded. For prevention of heat injury, the cut-off time of the test was 30 sec.

2.2.5. *Antidepression tests*

The tail suspension test: Mice were hung by their tail on the tail hanger using sticky tape for tail fixation, at approximately 1 cm from the end. The hanger was fixed in the black plastic box (20×20×45 cm) with the opening at the top front. The distance between the hangers to the floor was approximately 40 cm. The mouse was suspended in the air by its tail and the immobile time was recorded during the period of 5 min. The duration of immobility was defined as the absence of all movement except for those required for respiration.

The forced swim test: The forced swim test was carried out in a glass cylinder (20 cm diameter, 30 cm height) filled with water to the height of 20 cm. The water temperature was approximately 25-28°C. Mice were gently placed into the water and the immobility time was recorded by an observer during the period of 5 min. Immobility was defined as absence of all movement and remained floating passively in the water with its head just above the water surface.

2.2.6. *Motor activity tests*

The rota-rod test: Mouse was placed on the rotating drum with the acceleration speed (Acceler. Rota-rod, Jones & Roberts, for mice 7650, Ugo Basile, Italy). The time animal spent on the rod is recorded.

The wire mesh grasping test: The mouse was placed on a wire mesh (20×30 cm). After a few seconds, the mesh was turned 180° and the time the animal hold on the mesh was recorded.

2.2.7. *Statistical methods*

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

3. Results and Discussion

3.1. *Synthesis*

The diphenyl-pyrazolone template **1** was synthesised (*23*) in a condensation reaction by direct heating of diphenyl-hydrazine and ethylacetoacetate (*24*). Nitrosation and subsequent reduction served the building block for the preparation of amides and ureas.

Nitrosation of **1** with sodium nitrite furnished a nitroso-intermediate, which was reduced *in situ* with a solution of $SnCl_2$ in hydrochloric acid to give amine **2**. The nitroso-intermediate was found unstable, but may be isolated as green crystals.

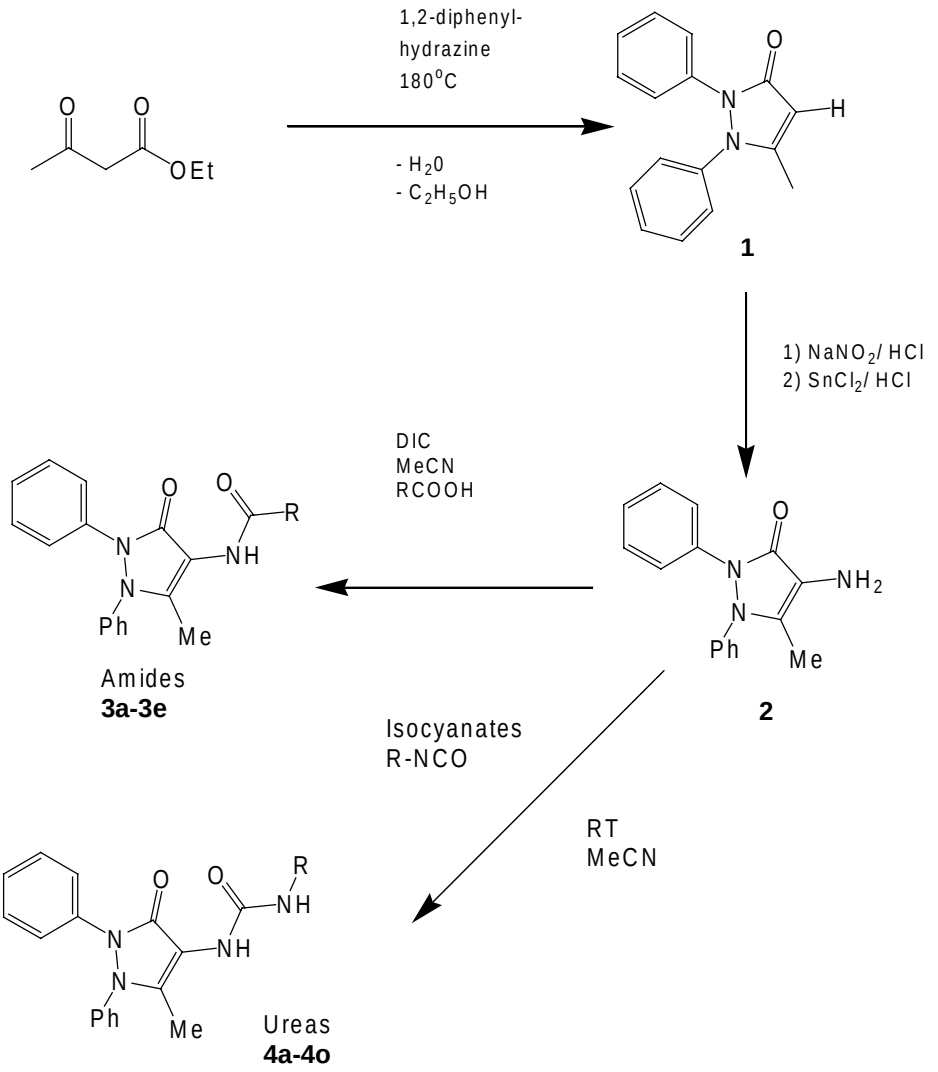
The nucleophilic amino group of the pyrazoline **2** was reacted with the DIC activated series of indole carboxylic acids giving the amido-pyrazolines **3a**-**3e**. For the preparation of diphenyl ureas **4a**-**4e** the amine **2** was reacted with the appropriate isocyanate to give the desired compounds as white solids in high yields (Scheme 1).

No purification by column chromatography was required for this chemical approach and selected spectroscopic data of diphenyl pyrazolyl indole carboxamides and ureas were reported in the experimental section. Other known intermediates and targets were reported by Farghaly (*25*).

3.2. *SAR studies / receptor binding affinity*

The diphenyl pyrazoline derivative of 2-indole carboxylic acid **3a**, in which the 1,4-benzodiazepine moiety of the known cholecystokinin ligands, was replaced by a pyrazoline template, showed a binding activity with an IC_{50} of 20 nM for the CCK_1 receptor, but a very poor solubility in water and organic solvents ($CHCl_3$, DMSO, MeCN) it could therefore, not fully characterised nor be tested *in vivo*.

Analogue **3b** of the diphenyl template, derived from 3-indolylcarboxylic acid, occurred a better solubility than **3a**, but showed a low binding affinity. A series of homologues containing a C1 (**3c**) and a C2 spacer unit (**3d**) displayed a CCK_1 selective binding affinity of 20 and 25 nM, respectively. The introduction of a C3 unit resulted in a loss of binding affinity for the derivative **3e**.



Scheme 1. Synthesis of pyrazolone based cholecystokinin-antagonists.

In a SAR optimisation of the ureido-diphenylpyrazoline template, it was found that *m*-substituted phenylureas generally displayed the highest binding affinity. The *o*-methoxyphenyl isocyanate gave poor yields for the **4a**, and was also biologically inactive. Only the *o*-derivative containing a nitro group was formed in good yield.

Compound **4e**, a meta-toluidine urea displayed an IC_{50} of 25 nM for the CCK_2 and 20 nM towards the CCK_1 receptor subtype. Approximately the same binding profile was determined for the *m*-methoxyphenylurea **4b**, which was selected for further evaluation. However, ureas containing ortho and para toluidine substituents showed generally no binding activity. Compound **4h**, containing a para-chlorine group, showed no activity, in line with the *p*-methyl and *p*-methoxy ureas **4c** and **4f**.

The cyclohexyl urea **5n** exhibited a modest binding affinity of ~1 μM. The unsubstituted phenyl urea **4l** and the aromatic naphthylurea (**4m**) analogues were found of only micromolar binding affinity (Table 1).

The first step of the evaluation was the determination of the receptor binding affinity for the CCK_1 and CCK_2 subtype, followed by an *in vivo* evaluation (*26*).

The highest MPE (maximum possible effect) value for antinociception, in conjunction with morphine, was obtained for the CCK_1 selective amide **3d** named **MPP** and it was tested further *in vivo*.

In the ureido series phenylurea **4b** and **4e**, ligands of the same binding profile, displayed the same high MPE. The mixed CCK antagonist, urea **4b**, was selected for further evaluation, due to the highest mixed binding affinity, the best clog p value and the highest chemical yield. In further investigations **4b** was named **MPM** (MPE data not shown, Figure 2).

3.3. *Antidepressant-like effects of amide **MPP** and urea **MPM***

Antidepressant drugs have the effect of reducing the duration of immobility in the despair swim test (immobility time test) (*27*). Desipramine, a tricyclic antidepressant served as positive control, which was found less potent, but has shown a similar magnitude of

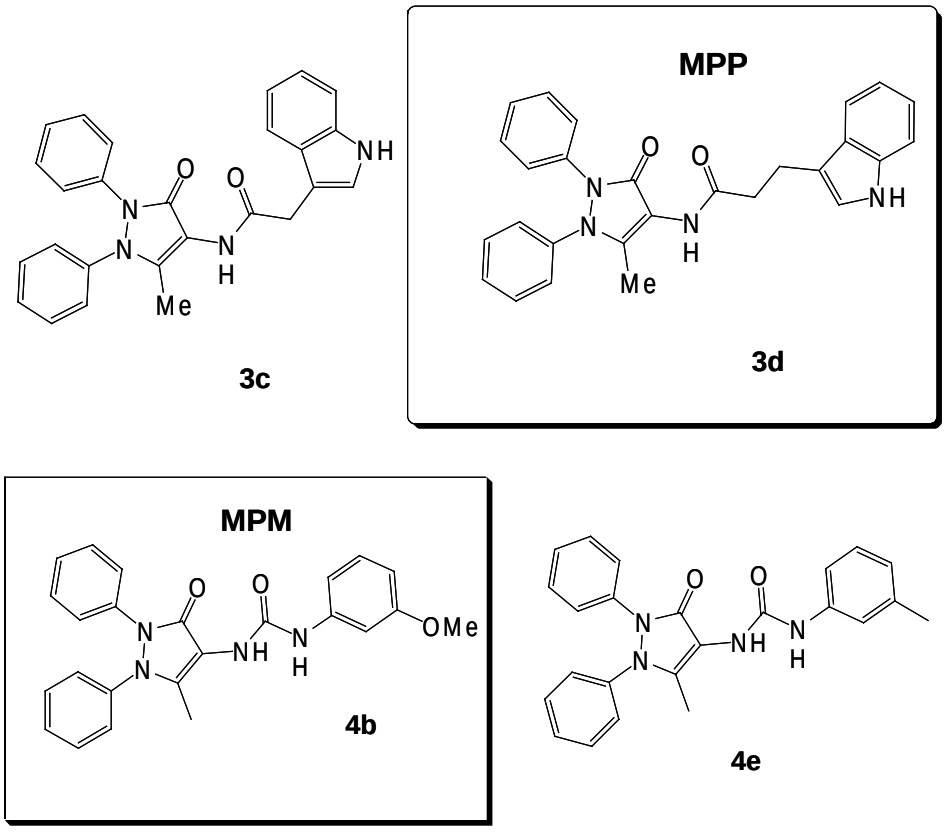


Figure 2. Selected structures of *N*-(3-oxo-2,3-dihydro-1*H*-pyrazol-4-yl)-1*H*-indole-carboxamides and ureido-pyrazolines.

the antidepressant effect.

Mice were intraperitoneally injected with 5% DMSO or antagonists **MPM** or **MPP** at various doses either 0.005, 0.01, 0.05 or 0.5 mg/kg BW or desipramine (10 mg/kg). The immobility times of the animals in each treatment group were shown in Figures 3 and 4.

MPM showed a significant antidepressant-like effect from 0.01 mg/kg BW and at higher doses (Figure 3). No dose-dependent effect of the CCK antagonists were seen and the effects of **MPM**, at all doses (except 0.005 mg/kg BW of **MPM**), were comparable to 10 mg/kg BW of desipramine.

MPP showed a significant antidepressant-like effect at all doses tested (0.005, 0.01, 0.05 and 0.5 mg/kg BW) and the antidepressant-like effect of desipramine was clearly observed (Figure 4).

In this study, the CCK_1 selective and the mixed CCK antagonist showed antidepressant-like effects supporting the roles of a CCK system in depression. The pharmacological effects seemed to be all-or-none without a dose-dependent property (*28*).

3.4. *Anxiolytic-like effects*

Animals have been evaluated in the black and white test (*29*) and the elevated plus maze test (x-maze)

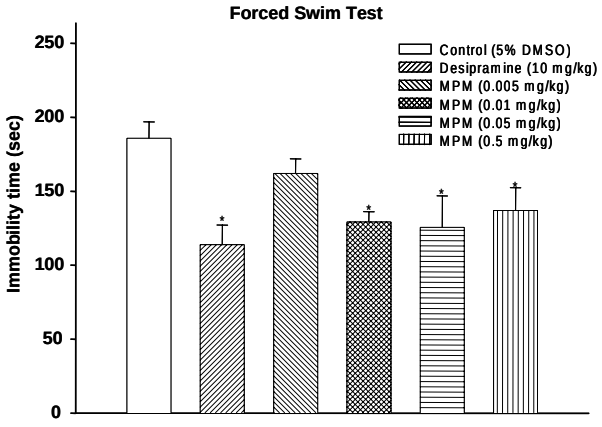


Figure 3. Dose-effect relationship of **MPM** on the immobility time of mice in the forced swim test.

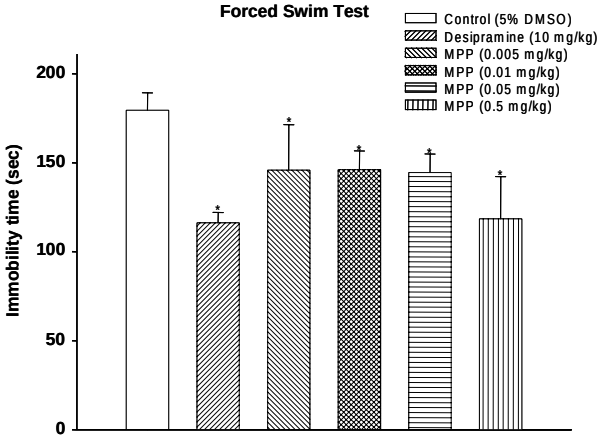
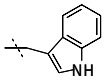
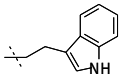
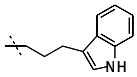


Figure 4. Dose-effect relationship of **MPP** on the immobility time of mice in the forced swim test.

Table 1. Structure and binding affinity of *N*-(5-methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-*N*'-phenylureas pyrazol-4-yl amides

Entry	Group R	Yield [%]	IC_{50} CCK_B [μM]	IC_{50} CCK_A [μM]
3a		65	2.1 ± 0.1	0.020 ± 0.001
3b		78	3.5 ± 0.4	2.2 ± 0.2
3c		66	2.5 ± 0.2	0.020 ± 0.002
3d		79	2.4 ± 0.2	0.025 ± 0.002
3e		80	20 ± 1	20 ± 1
4b	*m*-MeOPh	71	0.025 ± 0.002	0.010 ± 0.002
4c	*p*-MeOPh	76	> 20	9
4e	*m*-MePh	91	0.025 ± 0.002	0.020 ± 0.002
4f	*p*-MePh	89	> 20	13
4g	*m*-ClPh	73	> 20	7
4h	*p*-ClPh	81	> 20	> 20
4i	*p*-BrPh	92	> 20	> 20
4j	*o*-NO_2Ph	65	7.5	> 20
4k	*p*-NO_2Ph	77	> 20	> 20
4l	Ph	91	3	3
4m	Naphtyl	75	> 20	8
4n	Cyclohexyl	86	0.85 ± 0.2	12
4o	*t*-Bu	60	11	> 20

(*30*). In the elevated plus maze test a greatly enhanced exploration of the open arms with an increased number of total crossings was observed for the mixed antagonist **MPM**.

Mice were intraperitoneally injected with 5% DMSO or **MPM** or **MPP** at various doses either 0.005, 0.01, 0.05 or 0.5 mg/kg BW or diazepam (1 mg/kg) as positive control.

The anxiolytic-like effect of **MPM** (Figure 5) was observed at 0.05 and 0.5 mg/kg BW and no difference among the **MPM** (0.05 and 0.5 mg/kg BW) and diazepam-treated group could be seen. The 2 lower doses were not significantly different from the control. Thus, 50 μg/kg of the CCK antagonist **MPM** displayed the same anxiolytic effect as 1 mg/kg of the benzodiazepine ligand diazepam in mice. At a dose of 10 mg/kg **MPM** no impairment of coordination was found, which is associated with higher doses of the benzodiazepine anxiolytics.

MPP-treated groups were inactive in the x-maze test and the light and dark box test. No anxiolytic-like effect of **MPP**, the CCK_1 selective antagonist was obtained. Evidence indicated that CCK_1 receptors were involved in the mediation of anxiolytic-like effects in the elevated x-maze (*31*). In the same model CCK_2 antagonists also showed anxiolytic-like effect (*32*).

Thus, both CCK_1 and CCK_2 receptors antagonists

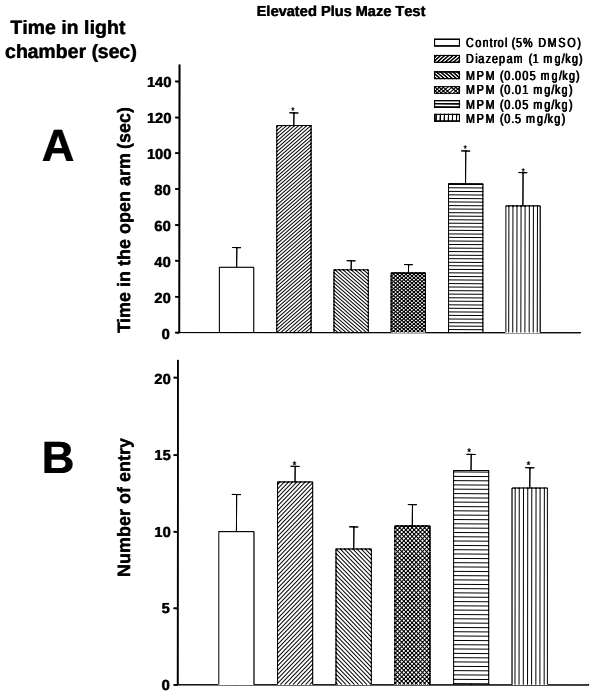


Figure 5. Dose-effect relationship of **MPM** on time in the open arms (A) and number of entry (B) in the elevated plus maze test.

in conjunction seem to have roles in the modulation of anxiety-related behaviour in animal models.

3.5. *Potentiation effect of **MPM** and **MPP** on morphine-induced analgesia in mice*

Nociception and motor activity tests: In all treated groups, no effect on nociception (*33*) was observed in the tail immersion test (*34*) and the hot plate method (*35*) up to 10 mg/kg for the test compounds as single agents. An impairment of motor activity could not be observed in all tested models up to a dose of 10 mg/kg in the wire mash grasping and the rota rod test.

MPM and **MPP** were selected to study the potentiation of morphine antinociception at a dose of 0.5 mg/kg body weight. From previous experiments fully developed biological effects were observed at this relatively high dose.

DMSO (5%), **MPM** or **MPP** (in 5% DMSO) was intraperitoneally injected as the first injection. Twenty min after the first, the second injection was subcutaneously injected with morphine at either 2, 4, 8 or 16 mg/kg BW. The thermal response latency of the animals was determined by the tail immersion test and the results were expressed as % MPE. Although no intrinsic analgesic effect of **MPM** and **MPP** could be observed, both CCK antagonists increased % MPE in response to morphine, at all tested morphine doses.

The morphine potentiation of **MPP** (0.5 mg/kg) and **MPM** (0.5 mg/kg) was determined at 60 and 90 min for a dose range of morphine and the results were outline in Figures 6A and 6B.

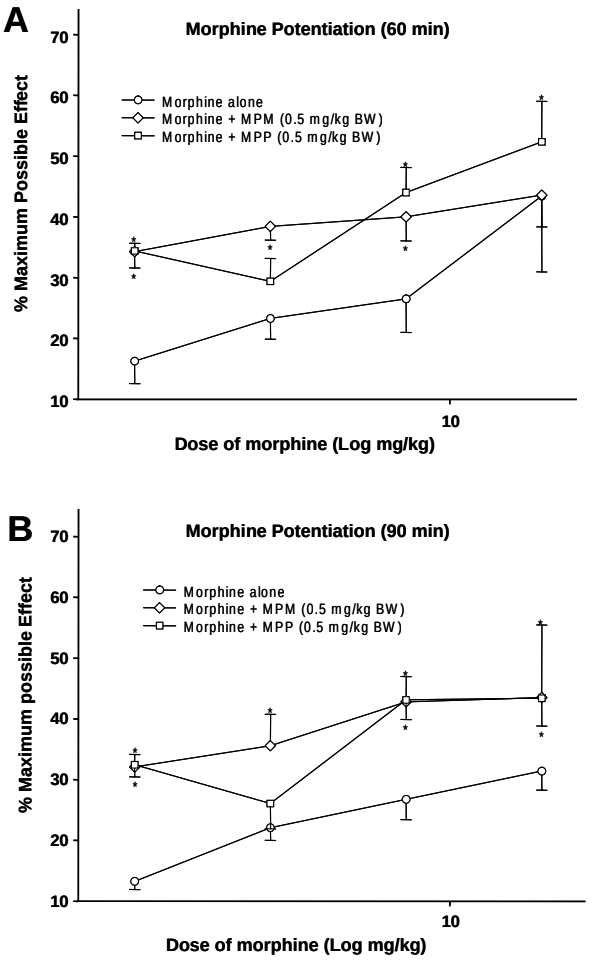


Figure 6. (A), Potentiation effect of **MPM** or **MPP** on morphine-induced analgesia at 60 min after morphine injection in mice; (B), Potentiation effect of **MPM** or **MPP** on morphine-induced analgesia at 90 min after morphine injection in mice.

In general, at a certain dose of morphine, there was no difference of MPE between the **MPM** and **MPP** groups. The potentiation was higher for low doses, such as 2 mg/kg of morphine and less for high doses, such as 16 mg/kg of morphine. In the middle range (4 mg/kg of morphine) the mixed antagonist **MPM** at a fixed dose of 0.5 mg/kg seemed to be superior to the CCK_1 selective **MPP**, but for final conclusions more data are required.

The effects of cholecystokinin on the modulation of pain transmission and the anti-opioid effects of cholecystokinin were well documented (*36*). CCK antagonists potentiated the antinociceptive effects of morphine and could also block the development of morphine tolerance (*37*).

4. Conclusions

The pyrrazol lead structure had been previously discovered in a chemically diverse library and subsequent SAR optimization was completed in the classical manner for a series of amides and phenylureas. The 2-phenyl pyrrazolone ring system and the meta-substituents on the *N*-phenylurea moiety were found essential for a potent cholecystokinin antagonism.

Our diphenyl-pyrrazoline template contained no chiral centre in the molecule. Merck's ureas had to be separated into enantiomeres, as the CCK_1/CCK_2 selectivity was dependent on the stereochemistry of the C3 center.

The importance of the removal of the stereo center was even greater, when it was found (*38*) that the one isomer of a 1,5-benzodiazepine urea acted as agonist the other as antagonist.

Lilly's diphenylpyrazolidinones had 2 chiral centres and after undergoing clinical trials, compound LY288513 was discontinued due to major adverse effects (*39*). The pyrrazoline template used here, displayed anti-inflammatory properties. From current animal experiments, there were no signs of acute toxicity and no long term toxicity (part 2).

These novel phenyl ureas represent CCK antagonists with a mixed binding profile and a potency in the nanomolar range for both receptor subtypes. Merck and other major pharmaceutical companies developed selective, mainly CCK_2 selective antagonists. The results of our animal experiments do not justify this approach, as a better pharmacological profile of our mixed antagonists was obtained.

The anxiolytic properties of the mixed antagonists were not associated with the side effects of benzodiazepines, acting on the $GABA_A$ receptor (*40*). Antidepressant effects were observed for both, the amido- and phenyl urea-pyrrazolines.

In terms of analgesia, a tenth of the morphine dose was required for the same antinociceptive effect in conjunction with a small dose of the CCK antagonist.

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