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Synthesis and evaluation of N-(3-oxo-2,3-dihydro-1*H*-pyrazol-4-yl)-1*H*-indole-carboxamides as cholecystokinin antagonists

Eric Lattmann, Harjit Singh, Yodchai Boonprakob, Pornthip Lattmann and Jintana Sattayasai

Abstract

The structure–activity relationship optimization of the pyrazoline template **3a** resulted in novel 3-oxo-1,2-diphenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-indole carboxamides **4a–4e**. These non-peptidal CCK ligands have been shown to act as potent CCK_1 ligands in a $^{[125]}$I-CCK-8 receptor binding assay. The best amides (**4c** and **4d**) of this series displayed an IC50 of 20/25 nM for the CCK_1 receptor. In a subsequent in-vivo evaluation using various behaviour pharmacological assays, an anxiolytic effect of these novel 3-oxo-1,2-diphenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-indole carboxamides was found at high doses in the elevated plus-maze. In the despair swimming test, a model for testing antidepressants, an ED50 of 0.33/0.41 mg kg^{-1} was determined for amide **4c/4d** and the antidepressant effect had a magnitude comparable to desimipramine.

Introduction

Cholecystokinin, which act as a neuromodulator/gut hormone, and CCK ligands, agonists as well as antagonists (McDonald 2001), have been extensively investigated as potential drug targets (Bock et al 1994; Masato et al 1995). CCK antagonists were studied as growth inhibitors in certain forms of cancer (Lattmann & Arayarat 2003), as anxiolytics (Dourish 1990; Hughes et al 1990), in the treatment of schizophrenia (Rasmussen et al 1995), satiety (Dourish et al 1989) and as antipanic agents (Francesco 1993; Trivedi & Bharat 1994). An agonist, the shortened CCK tetrapeptide, was found to induce panic in patients (Bradwejn et al 1990). A phase II trial of devazepide, a potent and CCK_1 selective antagonist (Evans et al 1988), has recently been completed (Simpson et al 2001), showing a significant enhancement of the effect of morphine in the treatment of chronic and severe pain (Hughes & Woodruff 1992).

Asperlicin, a microbial metabolite, was the first non-peptidal CCK antagonist and analogues thereof were studied as CCK ligands (Lattmann et al 2001). 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 (Figure 1). The 1,4-benzodiazepine template was varied by a combinatorial solid phase synthesis (Lattmann et al 2002a) and was optimized in terms of CCK binding affinity (Lattmann et al 2002b). In the search for new CCK ligands, in which the 1,4-benzodiazepine structure was replaced by an achiral template, the tryptophan-indole moiety was selected as the starting point (Figure 1).

The combination of indole carboxylic acids and amino-pyrazolines resulted in the discovery of a potent lead structure and the results are reported in this communication.

Traditionally, the pyrazoline template has been used for antipyretic, antirheumatic and analgesic drugs (Roth & Kleemann 1999).

Having realized the relevance of the CCK_1 receptor in the treatment of pain (Yu et al 2004) and depression (Lattmann et al 2005), indole amides of the pyrazoline template were prepared by a short synthetic approach and evaluated in receptor binding assays. Subsequently, the most potent ligands were tested in-vivo in mice.

The School of Pharmacy, Aston University, Aston Triangle, Birmingham B4 7ET, UK

Eric Lattmann, Harjit Singh, Pornthip Lattmann

Department of Pharmacology, Faculty of Medicine, Khon Kaen University, 40002 Khon Kaen, Thailand

Yodchai Boonprakob, Jintana Sattayasai

Correspondence: E. Lattmann, Lecturer in Medicinal Chemistry, The School of Pharmacy, Aston University, Aston Triangle, Birmingham B4 7ET, UK. E-mail: e.lattmann@aston.ac.uk

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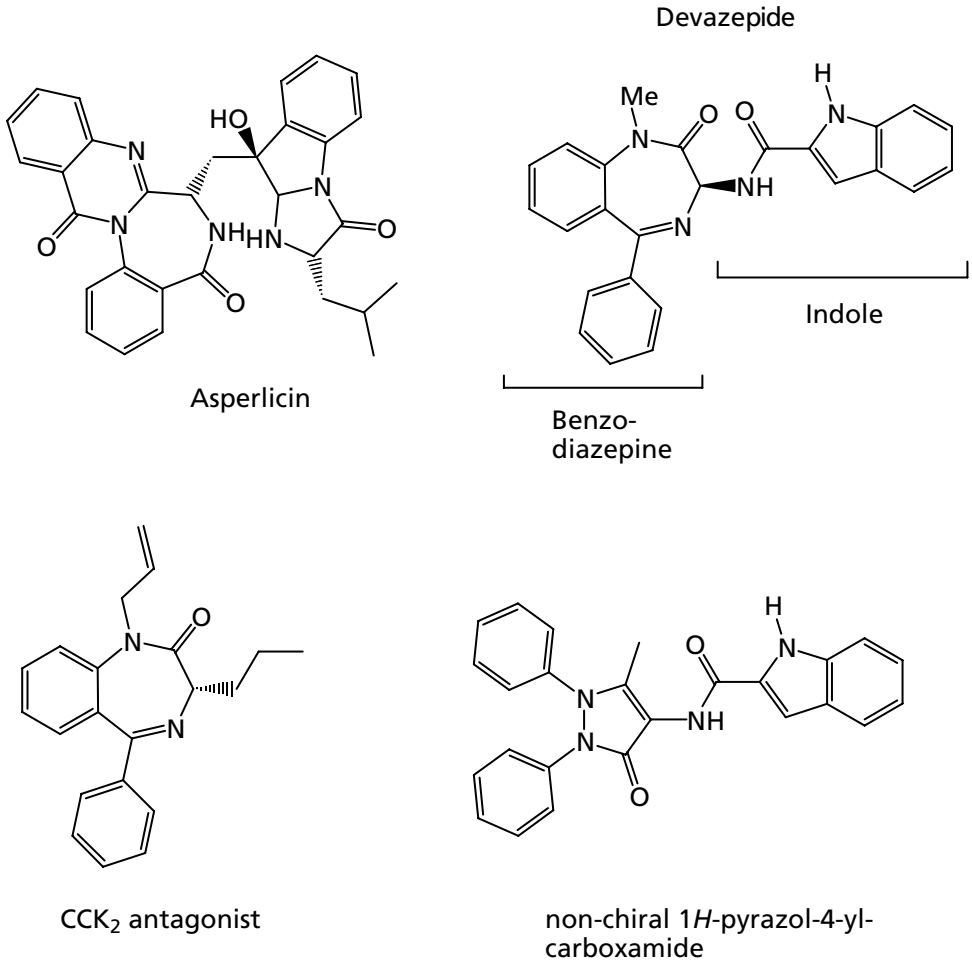


Figure 1 Development of cholecystokinin ligands.

Materials and Methods

Chemistry

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 using a Stuart Scientific (Coventry, UK) melting point apparatus and were uncorrected. Small-scale solution syntheses were carried out on a carousel reaction station (RR 98030), with a 12-place carousel reaction station and reflux head, and 12× flexible tubing from Radleys (Essex, UK).

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

Diphenyl hydrazine (50.0 g, 0.27 mol) and aceto ethyl acetate (2 equiv. 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/2 mmHg. This removed any unreacted diphenyl hydrazine to give a viscous black liquid. The mixture was allowed to cool to room temperature 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) ν_{max}: 3465, 3090, 1671, 1590, 1490, 1380, 1349, 1241, 971, 753 & 688 cm^{-1}. ^{1}H NMR ($CDCl_3$) 300 K δ: 2.07 (s, CH_3), 5.55 (s, CH), 7.05–7.37 (m, Ar–10H) ppm. ^{13}C NMR ($CDCl_3$) 300 K δ: 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) ppm.

*Synthesis of 4-amino-5-methyl-1,2-diphenyl-1,2-dihydro-3*H*-pyrazol-3-one* ***2b***

This compound was prepared via the synthesis of 4-nitroso-5-methyl-1,2-diphenyl-1,2-dihydro-3*H*-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 while stirring. A green precipitate was produced, which was allowed to stand for 45 min then filtered, washed with cold water and reacted further to the amine **2b**.

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 room temperature 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) ν_{max}: 3407, 3210, 1654, 1592, 1492, 1351, 1262, 751 & 690 cm^{-1}. ^1H NMR (DMSO-d_6) 300 K δ: 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) ppm. ^{13}C NMR (DMSO-d_6) 300 K δ: 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) ppm.

*Synthesis of N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-1*H*-indole-carboxamides*

General method: A solution of 4-amino-antipyrine (0.5 g, 2.45 mmol) was dissolved in dry acetonitrile (20 mL). The appropriate indole acid (1.25 equiv.) was added, with diisopropylcarbodiimide (DIC, 3 equiv.). The mixture was heated to 60°C and left overnight. The resulting precipitated crystals were filtered, washed and dried.

*N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-1*H*-indole-3-carboxamide* ***3a*** Yield: 644 mg, 76%. Mol. weight: 346.4. MS (APCI(+)): 347 (M + 1), 329 (M+) m/z. IR (KBr disc) ν_{max}: 3337, 3307, 2965, 1696, 1696, 1623, 15557, 1363, 1251 & 826 cm^{-1}. ^1H NMR (DMSO-d_6) 300 K δ: 2.18 (s, CH_3), 3.10 (s, N–CH_3), 5.50 (s, C = CH–), 7.02–7.06 (t, Ar–H, J = 7.4 Hz), 7.18–7.22 (t, Ar–H, J = 7.2, 7.3 Hz), 7.33–7.51 (m, Ar–6H), 7.62–7.65 (d, Ar–H, J = 8.0 Hz), 9.51 (s, NH), 11.56 (s, NH) ppm. Anal. calc. for $C_{20}H_{18}N_4O_2$: C, 69.35; H, 5.24; N, 16.17; O, 9.24. Found: C, 69.40; H, 5.25; N, 16.19; O, 9.16.

*N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-3-(1*H*-indol-3-yl)-propanamide* ***3d*** Yield: 687 mg, 75 %. Mol. weight: 374.4. Mol. formula: $C_{22}H_{22}N_4O_2$.MS (APCI(+)): 375 (M + 1) m/z. IR (KBr disc) ν_{max}: 3421, 3311, 3059, 2843, 1676, 1645, 1543, 1529, 1487, 1395, 1240 & 704 cm^{-1}. ^1H NMR (DMSO-d_6) 300 K δ: 2.01 (s, CH_3), 2.61–2.69 (t, CH_2, J = 8.1, 7.9 Hz), 3.02 (s, N–CH_3), 3.57–3.74 (m, CH_2), 6.93–7.58 (m, Ar–9H), 8.22–8.26 (d, Ar–H, J = 7.8 Hz), 9.08 (s, NH), 10.76 (s, NH) ppm. ^{13}C NMR (DMSO-d_6) 300 K δ: 22.2 (CH_3), 20.8 (CH_2), 23.8 (CO–CH_2), 36.5 (N–CH_3), 108.3 (C–NH), 111.9, 118.7, 122.5, 122.8, 123.9, 126.7 (2×C), 127.5, 127.6 (2×C), 129.6, 135.6, 136.7 (Ar–C), 162.4, 170.2 (C = O) ppm.

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

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 mmol) was dissolved in dry acetonitrile (20 mL). The appropriate indole acid (1.25 equiv.) was added with DIC (3 equiv.). 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-1*H*-pyrazol-4-yl)-1*H*-indole-2-carboxamide* ***4a*** 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) ν_{max}: 3401, 3339, 2965, 2358, 1710, 1615, 1583, 1454, 1361, 1172 & 748 cm^{-1}.

*N-(5-methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-1*H*-indole-3-carboxamide* ***4b*** 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) ν_{max}: 3343, 2965, 1615, 1581, 1535, 1494, 1453, 1318, 1249, 1191 & 750 cm^{-1}. ^1H NMR (DMSO-d_6) 300K δ: 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) ppm. ^{13}C NMR (DMSO-d_6) 300K δ: 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) ppm.

*N-(5-methyl-3-oxo-1,2-diphenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-2-(1*H*-indol-3-yl)acetamide* ***4c*** Yield: 211 mg, 66%. Mol. weight: 422.5. MS (APCI(+)): 423 (M + 1) m/z. IR (KBr disc) ν_{max}: 3337, 2965, 1679, 1648, 1629, 1592, 1525, 1488, 1312, 1243 & 749 cm^{-1}. ^1H NMR (DMSO-d_6) 300K δ: 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) ppm. ^{13}C NMR (DMSO-d_6) 300K δ: 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) ppm. Anal. calc. 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-1*H*-pyrazol-4-yl)-3-(1*H*-indol-3-yl)propanamide* ***4d*** Yield: 261 mg, 79%. Mol. weight: 436.5. MS (APCI(+)): 375 (M + 1) m/z. IR (KBr disc) ν_{max}: 3436, 3284, 1640, 1590, 1548, 1490, 1459, 1317 & 753 cm^{-1}. ^1H NMR (DMSO-d_6) 300 K δ: 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) ppm. ^{13}C NMR (DMSO-d_6) 300 K δ: 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) ppm. Anal. calc. 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-1*H*-pyrazol-4-yl)-4-(1*H*-indol-3-yl)butanamide* ***4e*** 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) ν_{max}: 3235, 3046, 1656, 1635, 1590, 1544, 1494, 1432, 1276 & 699 cm^{-1}. ^1H NMR (DMSO-d_6) 300 K δ: 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) ppm. ^{13}C NMR (DMSO-d_6) 300 K δ: 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) ppm.

Pharmacology

Cholecystokinin binding assay

^{125}I-CCK-8 receptor binding assay: The CCK_1 and CCK_2 receptor binding assays were performed by using guinea pig pancreas or guinea pig cerebral cortex, respectively. For the CCK_2 assay membranes from male guinea pig brain tissues were prepared according to the modification described by Saita et al (1994). For the CCK_1 binding assay pancreatic membranes were obtained as described by Charpentier et al (1988). All the binding assays were carried out in duplicate with L-365260 and devazepide as internal standards.

In order to prepare the tissue the cerebral cortex was weighed after dissection and then homogenized in 25 mL ice-cold 0.32 M sucrose for 15 strokes at 500 rpm. It was then centrifuged at 1000 *g* (3000 rpm) for 10 min. The supernatant was centrifuged at 20 000 *g* (13 000 rpm) for 20 min. This pellet was redispensed in the required volume of assay buffer as defined below with five strokes of homogenizer at 500 rpm. The final tissue concentration was 1 g original weight to 120 mL buffer. The tissue was stored in aliquots at –70°C.

For the receptor binding assay the radio ligand (^{125}I-Bolton Hunter labelled CCK, NEN) and the drugs to be tested were incubated at 25 pM with membranes (0.1 mg mL^{-1}) in assay buffer containing 20 mM Hepes, 1 mM EGTA, 5 mM $MgCl_2$, 150 mM NaCl at pH 6.5 for 2 h at room temperature. The incubations were terminated by centrifugation. The membrane pellets were washed twice with water and bound radioactivity was measured in a γ-counter.

Animal studies

Experiments were conducted in male IRC mice obtained from the Animal House, Faculty of Medicine, Khon Kaen University. Each experimental group consisted of six to eight animals and the treatment procedures were approved by the ethical committee, Faculty of Medicine, Khon Kaen University (HO 2434-76) in accordance with current UK legislation.

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

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 compartment and two-thirds as a light compartment. A 40-W light bulb was used and positioned 10 cm above the centre of the light component. The animals could walk freely between the dark and light parts through the opening. The time animals spent in the light part during a 5-min interval was recorded. The mouse was considered to be in the light part when its four 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 the 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 four legs.

Nociception tests

The thermal tail-flick test The thermal response latency was measured by the tail-flick 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 the water, was recorded; the cut-off time was 10 s in order to avoid damaging the animal's tissue.

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. To prevent heat injury, the cut-off time of the test was 30 s.

Antidepression tests

The tail suspension test Mice were hung by their tails on the tail hanger using sticky tape for tail fixation, at approximately 1 cm from the end. The hanger was fixed in a black plastic box (20×20×45 cm) with the opening at the top front. The distance between the hanger and the floor was approximately 40 cm. The mouse was suspended in the air by its tail and the immobile time was recorded during a period of 5 min. The duration of immobility was defined as the absence of all movement except that 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 a 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 a period of 5 min. Immobility was defined as absence of all movement and floating passively in the water with the head just above the water surface.

Motor activity tests

The rota-rod test Mice were placed on a rotating drum with accelerating speed (Acceler, Rota-rod, Jones & Roberts, for mice 7650, Ugo Basile, Italy). The time the animals spent on the rod was recorded.

The wire mesh grasping test Mice were placed on a wire mesh (20 × 30 cm). After a few seconds, the mesh was turned through 180° and the time the animals held onto the mesh was recorded.

Statistical methods

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

Results and Discussion

Synthesis

Pyrazolone **1b** was synthesized (Ruhkopf 1940; Stenzl et al 1950) by direct heating of 1,2-diphenyl-hydrazine with ethylacetoacetate in a condensation reaction (Figure 2). Nitrosation of **1b** with sodium nitrite furnished a nitroso intermediate, which was reduced in situ with a solution of $SnCl_2$ in hydrochloric acid to give amine **2b**. 4-Aminoantipyrine **2a** is commercially available from Aldrich. The nitroso intermediate was found to be unstable and may be isolated as green crystals.

The nucleophilic amino groups of the pyrazolines **2a** and **2b** were reacted with the DIC activated series of indole carboxylic acids, giving the amidopyrazolines **3a**–**3e** and diphenyl derivatives **4a**–**4e** as white solids in high yields (Figure 2).

No purification by column chromatograpy was required for this chemical approach and the full spectroscopic data of potent **3a**, novel **3d** and the novel diphenyl indole carboxamides **4a**–**4d** were reported in the experimental section. Other known intermediates and targets were reported by Farghaly (1979).

Pharmacology

Receptor binding affinity, structure–activity relationship studies and in-vivo tests in mice

Compound **3a** is a 2-indole carboxylic acid derivative displaying an IC50 of 80 nM and it is chemically available in one

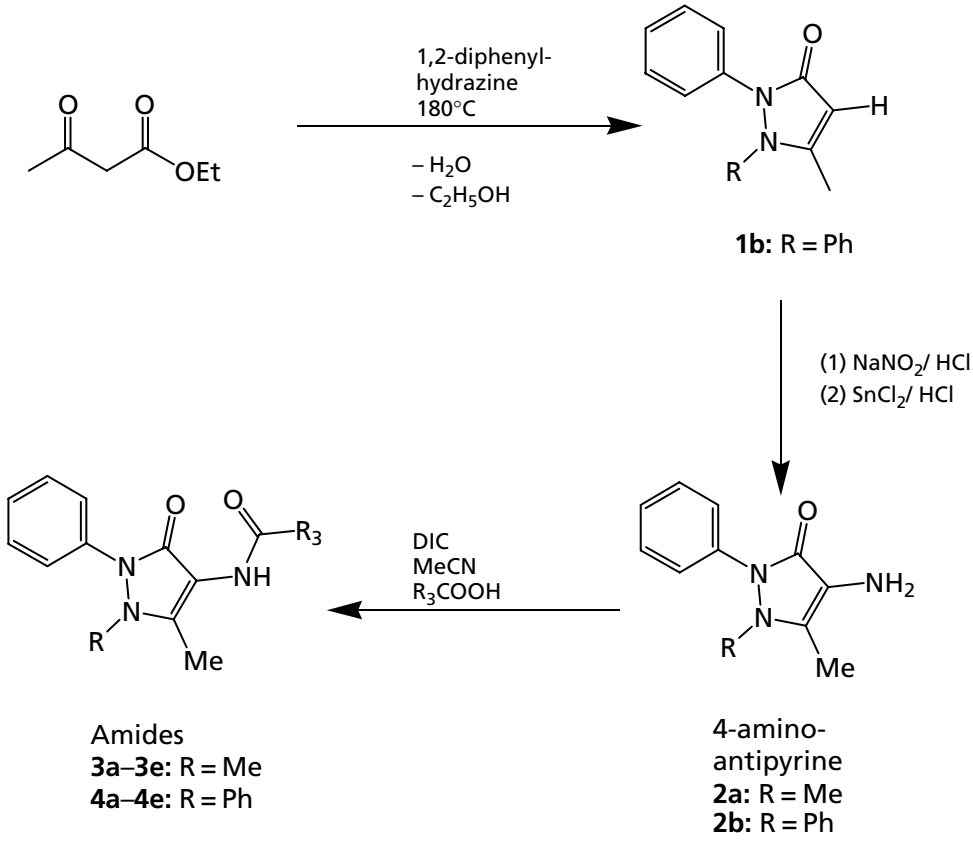


Figure 2 Synthesis of indole-carboxamides.

synthetic step from 4-amino-antipyrine. In-vivo tests of the test compound were performed stepwise at doses of 0, 0.1, 0.5, 1.0, 2.0, 5.0 and 10 mg kg^{-1}. For **3a** a minimum effective dose (MED) of 1.0 mg kg^{-1} was determined. Compared to **3a**, the diphenyl pyrazoline derivative of 2-indole carboxylic acid **4a**, in which the 1,4-benzodiazepine moiety of the known cholecystokinin ligands was replaced by a pyrazoline template, showed an enhanced binding activity with an IC50 of 20 nM for the CCK_1 receptor. Due to a very poor solubility in organic solvents ($CHCl_3$, DMSO, MeCN), it could only be tested in-vivo at a dose of 0.1 mg kg^{-1}.

Analogue **4b** of the diphenyl template, derived from 3-indolylcarboxylic acid, had better solubility than **4a**, but showed a low binding affinity (Table 1). A series of homologues containing C1 (**4c**) and C2 spacer units (**4d**) displayed CCK_1 selective binding affinities of 20 and 25 nM, respectively. The introduction of a C3 unit, such as in the derivative **4e**, resulted in a loss of binding affinity.

In Figure 3 the simplest derivative **3a** and the unsoluble CCK ligand **4a**, mimicking the binding interactions of devazepide in molecular modelling studies, are outlined. The 3-substituted indolyl derivatives **4c** and **4d** with a flexible spacer displayed a CCK_1 selective binding profile.

The first step of the in-vivo evaluation (Vogel & Vogel 1997) was the determination of the MED to select in-vivo active compounds and to compare the results with the receptor binding data. Pyrazolines **4c** and **4d**, ligands of the same binding profile, had MEDs of 0.1 mg kg^{-1} in both antidepressant assays. Compounds without binding affinity were not found to be different from the control (propylene glycol) at doses of 0.1, 0.5, 1.0, 20, 5.0 and 10 mg. For example, **4e** displayed no binding affinity and was found to be inactive in mice (Table 2).

Full data for the equipotent CCK_1 selective derivatives **4c** and **4d** were collected and the ED50 values were calculated (Table 3).

Anxiolytic assays

In the black and white test (Kilfoil et al 1989) animals showed a significantly increased preference for the light area at high doses, and the number of crossings between the two chambers was enhanced for **4c** and **4d**. An enhanced locomotor activity was determinated with an ED50 of 8.41/7.43 mg kg^{-1} for amide **4c/4d**. This correlated with the results of the elevated plus-maze test (x-maze) (Silverman 1978), in which a greatly enhanced exploration of the open arms with an increased number of total crossings was observed. The anxiolytic effect is supposedly due to a weak CCK_2 binding affinity. Compounds containing a urea linkage (Lattmann et al 2005) displayed similar anxiolytic and antidepressant effects to mixed CCK ligands at a similar low dose.

Antidepressant assays

Antidepressant drugs have the effect of reducing the duration of immobility in the despair swim test (immobility time test) (Porsolt et al 1977). The ligands **4c** and **4d** decreased the immobility time at a very low dose and the ED50 was calculated at 0.33 mg kg^{-1} for **4c** and 0.41 mg kg^{-1} for **4d**. In the tail suspension test, which is based on a similar underlying mechanism, an ED50 of 0.49/0.37 mg kg^{-1} was determined for **4c/4d**. Desipramine, a tricyclic antidepressant, served as positive control, which was found to be less potent but has shown a similar magnitude of antidepressant effect.

Nociception and motor activity tests

In all treated groups, no effect on nociception (Walker & Dixon 1985) was observed in the tail immersion test (Cowan 1990) and the hot plate method (Kitchen & Crowder 1985) up to 10 mg kg^{-1}. An impairment of motor activity could not be observed in all tested models up to a dose of 10 mg kg^{-1} in the wire mesh grasping and the rota-rod test.

Conclusions

Potent and novel CCK_1 ligands were developed in which the 1,4-benzodiazepine structure was replaced by a pyrazoline template. The synthetic approach, presented here, contained only three chemical steps. In step one the heterocyclic pyrazoline system was established, followed by nitrosation and reduction, which was carried out in situ. The reaction with DIC activated acids furnished the desired amides as stable and crystalline compounds.

As part of the structure–activity relationship optimization, the replacement of the 1-methyl-2-phenyl pyrazol-4-yl-system by a 1,2-diphenyl pyrazol-4-yl system, as well as the selection of the indole moiety with a spacer unit, were found to be essential for a high binding affinity.

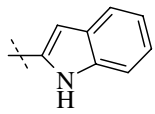
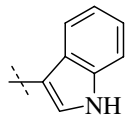
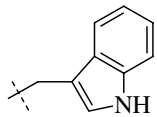
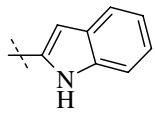
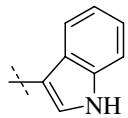
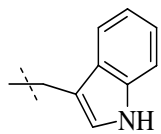
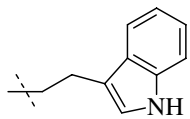
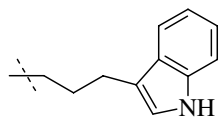
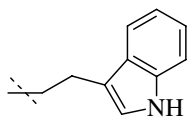
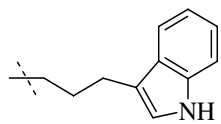
The pyrazoline compounds presented here contained no chiral centre in the molecule. Merck's ureas had to be separated into their enantiomeres as the CCK_1/CCK_2 selectivity was dependent on the stereochemistry of the C_3 centre. The removal of the chiral centre waived the separation of the reaction intermediates, thus shortening the chemical synthesis. The importance of the removal of the stereo centre was even greater when it was found (Hirst et al 1996) that the one isomer of a 1,5-benzodiazepine derivative acted as agonist with the other isomer as antagonist.

Lilly's diphenyl-pyrazolidinones had two chiral centres and after undergoing clinical trials compound LY288513 was discontinued due to major adverse effects (Rasmussen et al 1993). The pyrazoline template used here displayed anti-inflammatory properties and from further unreported animal experiments as part of pharmacokinetic and pharmacodynamic studies there are no signs of acute toxicity of our ligands.

These novel, structurally related CCK antagonists displayed a CCK_1 selective binding affinity. The anxiolytic properties, observed at relatively high doses, correlated with a weak CCK_2 binding affinity. Unlike the classical anxiolytics, acting on the $GABA_A$ receptor (Martin & Lattmann 1999), no signs of muscle relaxation and no impairment of coordination (rota-rod, wire mesh grasping) were observed for the four series.

A clear antidepressant effect was found for the amides **4c/4d** in two different standard assays and an ED50 of less than 0.5 mg kg^{-1} was determined. These findings initiated further work to establish the underlying biological mechanism of cholecystokinin in the treatment of depression.

Table 1 Structure–activity relationship of pyrazol-4-yl amides, receptor binding affinity

Entry cpd	Group R	Group R_3	Yield (%)	MW	IC50 CCK_2 (μM)	IC50 CCK_1 (μM)	Ratio CCK_2/CCK_1
3a	Me		76	346	0.9 ± 0.1	0.080 ± 0.006	11.3
3b	Me		80	346	15 ± 1	2.1 ± 0.4	7.5
3c	Me		82	360	9 ± 1	2.2 ± 0.3	4.5
4a	Ph		65	408	2.1 ± 0.1	0.020 ± 0.001	100
4b	Ph		78	408	3.5 ± 0.4	2.2 ± 0.2	1.8
4c	Ph		66	422	2.5 ± 0.2	0.020 ± 0.002	125
4d	Ph		79	436	2.4 ± 0.2	0.025 ± 0.002	80
4e	Ph		80	450	20 ± 1	20 ± 1	1
3d	Me		75	374	4 ± 1	1.1 ± 0.2	4
3e	Me		78	388	20 ± 2	20 ± 2	1

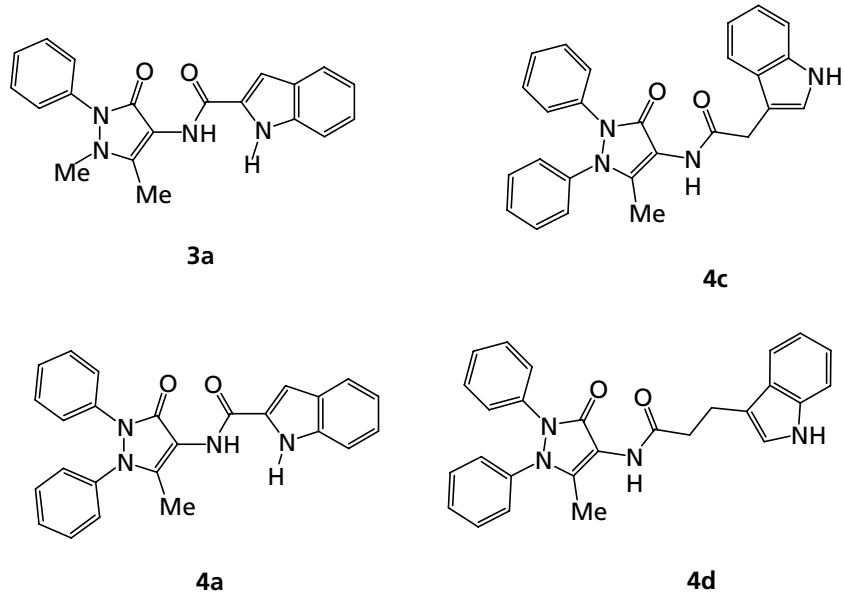


Figure 3 Selected structures of N-(3-oxo-2,3-dihydro-1*H*-pyrazol-4-yl)-1*H*-indole-carboxamides.

Table 2 In-vivo evaluation of selected N-(3-oxo-2,3-dihydro-1*H*-pyrazol-4-yl)-carboxamides

Compound	Receptor binding IC50 (nM)		Elevated plus-maze	Light/ dark box	Tail suspension test	Forced swim test	Thermal tail-flick test	Hot plate test	Rota-rod test	Wire mesh grasping test
	CCK_1	CCK_2								
3a	80 ± 6	900 ± 100	NS	NS	1.0	1.0	NS	NS	NS	NS
4a	20 ± 1	2100 ± 100	Ins.	Ins.	0.1	0.1	Ins.	Ins.	Ins.	Ins.
4c	20 ± 2	2500 ± 200	5.0	5.0	0.1	0.1	NS	NS	NS	NS
4d	25 ± 2	2400 ± 200	5.0	5.0	0.1	0.1	NS	NS	NS	NS
4e	20000 ± 1000	20000 ± 1000	NS	NS	NS	NS	NS	NS	NS	NS

NS, no significance could be observed at 0.1, 0.5, 1.0, 2.0, 5.0 and 10 mg kg^{-1} compared to the control; Ins, insoluble at higher test concentrations ≥ 0.1 mg kg^{-1}.

Table 3 In-vivo studies of selected CCK antagonists in mice

	ED50 (mg kg^{-1})	
	4c	4d
Elevated plus-maze	7.49 ± 0.15	8.61 ± 0.15
Light/dark box	8.41 ± 0.20	7.43 ± 0.20
Tail suspension test	0.49 ± 0.20	0.37 ± 0.12
Forced swim test	0.33 ± 0.21	0.41 ± 0.11
Thermal tail-flick test	>10	>10
Hot plate	>10	>10
Rota-rod test	>10	>10
Wire mesh grasping test	>10	>10

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