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Synthesis and evaluation of N_1-substituted-3-propyl-1,4-benzodiazepine-2-ones as cholecystokinin (CCK_2) receptor ligands

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Abstract

A novel synthetic approach towards N_1-alkylated 3-propyl-1,4-benzodiazepineswas developed in five synthetic steps from 2-amino-4-chlorobenzophenone, in which the N-oxide **4** served as a key intermediate. The structure–activity relationship optimization of this 3-propyl-1,4-benzodiazepine template was carried out on the N_1-position by selective alkylation reactions and resulted in a ligand with an improved affinity on the cholecystokinin (CCK_2) receptor. The *N*-allyl-3-propyl-benzodiazepine **6d** displayed an affinity towards the CCK_2 (CCK-B) receptor of 170 nM in a radiolabelled receptor-binding assay. The anxiolytic activity of this allyl-3-propyl-1,4-benzodiazepine **6d** was subsequently determined in in-vivo psychotropic assays. This novel ligand had ED50 values of 4.7 and 5.2 mg kg^{-1} in the black and white box test and the x-maze, respectively, and no significant sedation/muscle relaxation was observed.

Introduction

The peripheral effects of cholecystokinin (Trivedi & Bharat 1994) are mediated mainly through the A receptor subtype (CCK_1), while the central effects are correlated with the B receptor subtype (CCK_2) (Evans et al 1993). The CCK_2 receptor is involved in many pathological situations. Among these, anxiety (Bain & Candillis 2000) and panic are particularly relevant targets for therapeutic interventions (Tullio et al 2000). Sulfated CCK-5 is widely studied as an ideal panic-triggering agent. The best evidence that CCK is strongly related to panic attacks is based on experiments in which CCK-4 was administered to healthy volunteers who had panic attacks shortly after the injection (Wilson at al 1996). Those physiological reactions were significantly blocked or reduced by administrating Merck's CCK_2 selective antagonist, L-365,260, which is chemically a 3-ureido-1,4-benzodiazepine (Evans et al 1986) (Figure 1).

We have previously identified the *n*-propyl side chain in the 3-position of 1,4-benzodiazepines as the best substituent for CCK ligands by a solid phase combinatorial approach and included structure–activity relationship studies on the ketone moiety of this molecule (Lattmann et al 2001). Here, we report a synthetic approach towards the synthesis of this template **5** and the influence of the N_1-alkylation of the 3-propyl-1,4-benzodiazepine on the affinity at the CCK_2 receptor.

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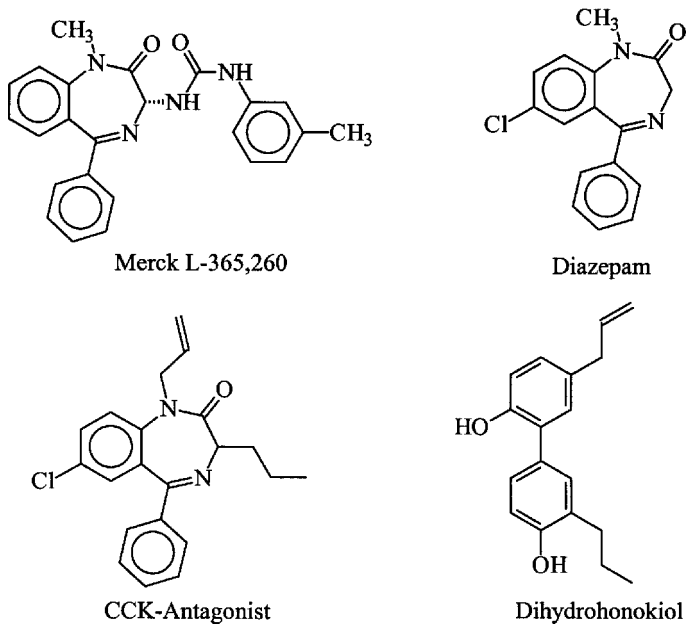


Figure 1 Selective antagonists.

Materials and Methods

Chemistry

Atmospheric pressure chemical ionization mass spectrometry (APCI-MS) was carried out on a Hewlett-Packard 5989B quadrupole instrument connected to a 59987A unit with an APCI accessory. IR spectra were recorded as KBr discs on a Mattson 3000 FTIR spectrophotometer. Proton NMR spectra were obtained on a Bruker AC 250 instrument operating at 250 MHz, with tetramethylsilane as internal standard.

2-Bromo-N-{*4-chloro-2-[(hydroxyimino)(phenyl)-methyl] phenyl*}*pentanamide* (***3***)

A solution of 0.10 mol (2-amino-5-chlorophenyl)-(phenyl)methanone oxime **2** and 0.12 mol 2-bromo-1-chloropentan-1-one in 200 mL 1,2-dichloroethane was refluxed for 3–4 h, cooled and washed with brine. The organic layer was separated, dried with anhydrous sodium sulfate, filtered and concentrated in-vacuo. The crystalline residue was recrystallized to give 70% of the title product: 409.7 g mol^{-1}.

APCI+m/s: m/z = 393 (50%, fragmentation, minus OH), 329 (30%), 313 (5%), 283 (15%); TLC (ether): R_f = 0.7; IR (KBr) ν_{max} = 3378, 2962, 2857, 1721, 1513, 1384, 1285, 1120, 1071, 695 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.0 (m, 3H, -CHBr$(CH_2)_2CH_3$), 1.25–2.0 (m, 4H, -CHBr$(CH_2)_2CH_3$), 4.25 (m, 1H, -C*H*Br$(CH_2)_2CH_3$), 7.0–8.0 ppm. (m, 8 arom. H and -N*H*CO).

*7-Chloro-2-oxo-5-phenyl-3-propyl-2,3-dihydro-1*H*-1,4-benzodiazepin-4-ium-4-olate* (***4***)

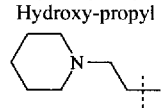
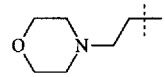
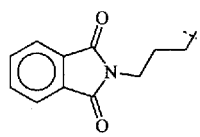
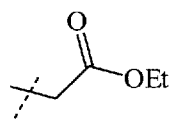
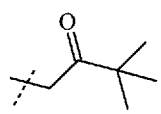
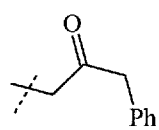
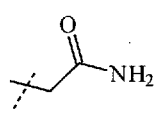
A solution (60 mmol) of **3** was stirred for 3–4 h at 40°C with a mixture of 400 mL ethanol and 125 mL 2 M sodium hydroxide. The solid that separated out was removed by filtration. The crude material was collected and recrystallized from a mixture of acetone and petroleum ether. A higher amount of the title compound was obtained by adding salt to the mixture. Recrystallization of the crude material with ethanol/petrolether gave the target molecule in 60% yield.

APCI+m/s: m/z = 329 (80%), 313 (20%); TLC (ether): R_f = 0.45; IR (KBr) ν_{max} = IR (KBr disc) ν_{max} = 3430, 3189, 3070, 2935, 1694, 1486, 1440, 1291, 1208 (N-oxide), 825, 688 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.0 (tr; 3H, J = 7 Hz, C3:-$(CH_2)_2CH_3$), 1.3–1.6 (m, 2H, C3: -$CH_2CH_2CH_3$), 2.1+2.5 (two br s, 2H, C3: -$CH_2CH_2CH_3$), 4.15 (tr, 1H, J = 5 Hz, C3:-*H*), 7.0–7.6 (m, 8 arom. H), 10.1 (s, 1H, N1:-H); ^{13}C NMR ($CDCl_3$) δ 14.0 (C3:-$(CH_2)_2CH_3$), 19.4 (C3:-$CH_2CH_2CH_3$), 36.9 (C3:-$CH_2CH_2CH_3$), 69.6 (*C*3:-$(CH_2)_2CH_3$), 123.2, 128.1, 129.9, 130.1, 130.5, 130.8, 131.5, 135.0, 136.4, 165.4, 170.1 ppm.

*Preparation of 7-chloro-5-phenyl-3-propyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one* (***5***)

To a suspension of 0.06 mol 3-propyl-benzodiazepine N-oxide **4** in 300 mL chloroform, 17.2 mL (0.2 mol) of phosphorus trichloride was added. The stirred mixture

Table 1 Chemical yields and IC50 (μM) receptor binding data on the cholecystokinin (CCK_2) receptor of the 1,4-benzodiazepines (standard: 10 nM L-365,260, Merck).

Compound	Reagent	R_1	Yield	CCK_2
5	–	H	-	0.3 ± 0.01
6a	MeI, RT	Me	65	0.5 ± 0.02
6b	Bromide, RT	Et	78	1.2 ± 0.6
6c	Bromide, RT	propyl	74	5 ± 2
6d	Bromide, RT	allyl	89	0.17 ± 0.02[a]
6f	Bromide	propargyl	84	0.2 ± 0.02
6g	Chloride	Cyano-methyl	53	0.19 ± 0.02
6h	Bromide	butyl	65	3 ± 1
6i	Bromide	i-butyl	54	12 ± 4
6j	Chloride	benzyl	65	35 ± 3
6k	Bromide	phenyl-ethyl	63	56 ± 6
6l	HCHO	hydroxy-methyl	21	37 ± 11
6m	2-Bromo-ethanol	Hydroxy-ethyl	43	76 ± 12
6n	3-Bromo-propanol	Hydroxy-propyl	31	71 ± 21
6o	Bromide		82	88 ± 12
6p	Bromide		54	74 ± 8
6q	Bromide		87	34 ± 6
6r	Chloride		63	3.5 ± 0.4
6s	Chloride		42	7 ± 1.5
6t	Bromide		21	4 ± 1.5
6u	Bromide		32	24 ± 5

[a]GABA-A binding > 50 m. RT indicates reaction carried out at room temperature.

was refluxed for 1 h and the solvent was removed under reduced pressure. To this residue, methylene chloride and a large excess of 50% potassium hydroxide were added. The aqueous layer was extracted three times with methylene chloride. The organic layers were combined, dried, filtered to remove a fine amorphous impurity and concentrated in-vacuo. The residue was recrystallized with ether yielding 80% of the desired molecule.

APCI+m/s: m/z = 313 (80%), 268 (15%), 264 (5%); mp: 197–198 °C; TLC (ether): R_f = 0.72; IR (KBr disc) ν_{max} = 3218, 3123, 2923, 2851, 1687, 1604, 1476, 1320, 1220, 826, 699 cm^{-1}; ^1H NMR ($CDCl_3$) δ ^1H NMR ($CDCl_3$) δ 0.99 (t; 3H, J = 7 Hz, C3:-$(CH_2)_2CH_3$), 1.3–1.5 (m, 2H, C3:-$CH_2CH_2CH_3$), 2.23 (m, 2H, C3: -$CH_2CH_2CH_3$), 3.53 (t, 1H, J = 5 Hz, C3:-*H*), 7.15–7.7 (m, 8 arom. H), 10.3 (s, 1H, N1:-H); ^{13}C NMR ($CDCl_3$) δ 14.5 (C3:-$(CH_2)_2CH_3$), 19.3 (C3:-$CH_2CH_2CH_3$), 33.1 (C3:-$CH_2CH_2CH_3$), 63.2 (C3:-$(CH_2)_2CH_3$), 122.8, 124.9,

129.1, 130.2, 130.8, 131.6, 135.7, 137.4, 168.0 (C-5) 172.5 ppm. (*C*═O).

General experimental for the N-alkylation of 3-propyl-benzodiazepines

The N_1-alkyl/aryl derivatives (Table 1) were obtained by deprotonating the parent amide **5** with 1.1 equiv. sodium hydride in *N*,*N*-dimethylformamide, followed by the reaction of the in-situ-formed anion with 1.2 equiv. of the electrophile. The alkylation towards the methyl, ethyl, propyl and butyl derivatives **6a**–**6d** was carried out at 20°C. The alkylation with the electrophiles giving the phthalimide, morpholino, piperidine derivatives **6o**–**6q** was carried out at 50°C (Table 1).

The N_1-hydroxylmethyl-benzodiazepine **6l** was obtained from formaldehyde, generated by thermal decomposition of paraformaldehyde. After 30 min, the reactions were quenched with 10% aqueous HCl. The mixture was extracted three times with methylen-chloride, concentrated in-vacuo to give the N_1-alkylated benzodiazepines. The crude product was purified further by solid extraction with ether.

*7-Chloro-1-methyl-5-phenyl-3-propyl-1,3-dihydro-2*H-*1,4-benzodiazepin-2-one (**6a**)*

APCI+m/s: m/z = 327; TLC (ether): R_f = 0.75; IR (KBr) ν_{max} = 2971, 2861, 1715, 1669, 1461, 1253, 1114, 1073, 738, 705 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.99 (t; 3H, J = 7 Hz, C3:-$(CH_2)_2CH_3$), 1.35–1.52 (m, 2H, C3:-$(CH_2)_2CH_3$), 1.99 (m, 2H, C3:-$CH_2CH_2CH_3$), 3.48 (s, 3H, N1:-CH_3); 4.17 (t, 1H, J = 5 Hz, C3:-*H*), 7.2–7.87 (m, 8 arom. H), ^{13}C NMR ($CDCl_3$) δ 14.1 (C3:-$(CH_2)_2CH_3$), 19.1 (C3:-$CH_2CH_2CH_3$), 28.9 (N1:-CH_3), 35.0 (C3:-$CH_2CH_2CH_3$), 68.0 (C-3), 122.6, 128.5, 129.5, 130.8, 131.3, 138.1, 141.2, 167.7, 171.7 ppm.

*1-Allyl-7-chloro-5-phenyl-3-propyl-1,3-dihydro-2*H-*1,4-benzodiazepin-2-one (**6d**)*

APCI+m/s: m/z = 353; TLC (ether): R_f = 0.85; IR (KBr) ν_{max} = 3062, 2954, 2867, 1671, 1602, 1557, 1482, 1403, 14, 1197, 1131, 921, 825, 699 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.73 (t, 3H, N1:-$CH_2CH_2CH_3$), 1.01 (t; 3H, J = 7 Hz, C3:-$(CH_2)_2CH_3$), 1.25-1.9 (m, 4H, C3:-$CH_2CH_2CH_3$), 3.52 (m, 2H, N1:-$CH_2CH{=}CH_2$), 4.56 (m, 1H, C3:-*H*), 5.06–5.13 (m, 2H, N1:-$CH_2CH{=}CH_2$), 5.8–5.85 (m, 1H, N1:-$CH_2CH{=}CH_2$), 7.25–7.6 (m, 8 arom. H); ^{13}C NMR ($CDCl_3$) δ 14.2 (C3:-$(CH_2CH_2CH_3$), 19.3, 33.6, 50.1, 63.5 (C-3), 117.1 (N1:-$CH_2CH{=}CH_2$), 123.3, 129.4, 129.8, 130.4, 131.3, 131.8, 132.9, 138.2, 141.2, 167.1, 169.4 ppm.

*7-Chloro-5-phenyl-3-propyl-1-prop-2-ynyl-1,3-dihydro-2*H-*1,4-benzodiazepin-2-one (**6f**)*

APCI+m/s: m/z = 351; TLC (ether): R_f = 0.87; IR (KBr) ν_{max} = 3295, 2954, 2867, 1691, 1608, 1478, 1407, 1324, 1193, 1133, 825, 685 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.01 (t; 3H, J = 7 Hz, C3:-$(CH_2)_2CH_3$), 1.2–1.8 (m, 4H, C3:-$CH_2CH_2CH_3$), 2.35 (m, 1H, N1:-$CH_2C{\equiv}CH$), 3.5 (m, 1H, C3:-*H*), 4.6 (m, 2H, N1:-$CH_2C{\equiv}CH$), 7.25–8.0 (m, 8 arom. H); ^{13}C NMR ($CDCl_3$) δ 10.1 (C3:-$(CH_2)_2CH_3$), 19.3 (C3:-$CH_2CH_2CH_3$), 33.5, 36.8, 63.3 (C-3)), 72.1, 122.9, 128.3, 129.5, 129.8, 130.5, 131.1, 138.2, 140.5, 167.3 (*C*═O), 169.6 ppm.

*2-(7-Chloro-2-oxo-5-phenyl-3-propyl-2,3-dihydro-1*H-*1,4-benzodiazepin-1-yl)acetonitrile (**6g**)*

APCI+m/s: m/z = 352 (90%), 268 (10%); TLC (ether): R_f = 0.81; IR (KBr) ν_{max} = 2930, 2861, 2280 (N1:-CH_2-*C*≡*N*), 1723, 1687, 1598, 1324, 1265, 825, 744, 695 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.01 (t, 3H, J = 7 Hz, C3:-$CH_2CH_2CH_3$), 1.1–1.9 (m, 4H, C3:-$CH_2CH_2CH_3$), 3.45+3.6 (two m, 2H, N1:-CH_2-C═N), 4.5 (q, 1H, J = 5Hz, C3:-*H*), 7.1–7.8 7.1–7.9 (m, 8 arom. H); ^{13}C NMR ($CDCl_3$) δ 14.1 (C3:-$CH_2CH_2CH_3$), 19.3 (C3:-$CH_2CH_2CH_3$), 33.4 (C3:-$CH_2CH_2CH_3$), 38.9 (N1:-CH_2-C≡N), 62.9 (*C*3:-H), 115.4 (N1:-CH_2-*C*≡N), 122.8, 128.4, 128.9, 129.4, 130., 130.9, 131.5, 137.4, 139.2, 167.4, 169.3 ppm.

*1-(*sec*-Butyl)-7-chloro-5-phenyl-3-propyl-1,3-dihydro-2*H-*1,4-benzodiazepin-2-one (**6i**)*

APCI+m/s: m/z = 369 (90%), 313 (10%); TLC (ether): R_f = 0.91; IR (KBr) ν_{max} = 2955, 2857, 1737, 1683, 1461, 1378, 1272, 1116, 1069, 960, 740, 695 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.85–1.9 (m, 15H, C3:-$CH_2CH_2CH_3$+N1:-$CH(CH_3)CH_2CH_3$), 4.2 (m, 2H, C3:-*H*+N1:-$CH(CH_3)CH_2CH_3$), 7.3–7.7 (m, 8 arom. H); ^{13}C NMR ($CDCl_3$) δ 11.5, 14.1, 18.7, 19.4, 28.8, 30.3, 38.6, 67.9 (C-3), 128.3, 128.7, 129.1, 130.4, 130.8, 132.4, 138.1, 140.9, 167.6, 169.1 ppm.

*7-Chloro-1-phenethyl-5-phenyl-3-propyl-1,3-dihydro-2*H-*1,4-benzodiazepin-2-one (**6k**)*

APCI+m/s: m/z = 417 (85%), 313 (5%), 279 (10%); TLC (ether): R_f = 0.91; IR (KBr) ν_{max} = 2957, 2924, 2857, 1717, 1683, 1461, 1283, 1121, 1069, 740, 695 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.98 (t, 3H, J = 7 Hz, C3:-$CH_2CH_2CH_3$); 1.45–1.75 (m, 4H, C3:-$CH_2CH_2CH_3$), 4.25 (m, 3H, C3:-*H* + N1:-CH_2-CH_2-Ph), 6.8–7.75 (m, 13 arom. H); ^{13}C NMR ($CDCl_3$) δ 4.1 (C3:-$CH_2CH_2CH_3$), 22.9 (C3:-$CH_2CH_2CH_3$), 30.1 (C3:-$CH_2CH_2CH_3$), 38.6, 68.1 (*C*3:-H), 123.9, 126.4, 126.9,

128.5, 128.7, 130.8, 132.3, 136.6, 139.3, 141.3, 167.2 (*C*═O), 168.8 ppm.

*7-Chloro-1-(2-hydroxyethyl)-5-phenyl-3-propyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (**6m**)*

APCI + m/s: m/z = 357 (65%), 313 (35%); TLC (ether): R_f = 0.59; IR (KBr) ν_{max} = 3428, 2954, 2867, 1675, 1579, 1405, 1326, 1076, 823, 685 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.97 (t, 3H, J = 7 Hz, C3:-($CH_2CH_2CH_3$); 1.1–2.3 (m, 4H, C3:-($CH_2CH_2CH_3$), 3.53 (t, 2H, J = 5 Hz, N1:-CH_2-CH_2-OH), 3.8–4.0 (m, 3H, N1:-CH_2-CH_2-O*H*), 4.2 (m, 1H, C3:-*H*), 7.2–7.6 (m, 8 arom. H); ^{13}C NMR ($CDCl_3$) δ 14.5 (C3:-$CH_2CH_2CH_3$), 19.3, 33.4 (C3:-$CH_2CH_2CH_3$), 51.5, 61.2 (C-3), 102.4, 124.3, 128.4, 129.4, 130.5, 131.4, 136.8, 141.8, 168.2, 170.1 ppm.

*7-Chloro-1-(2-morpholin-4-ylethyl)-5-phenyl-3-propyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (**6p**)*

APCI+m/s: m/z = 426; TLC (ether): R_f = 0.82; IR (KBr) ν_{max} = 3432, 2954, 2853, 1675, 1608, 1476, 1320, 1118, 817, 695 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.97 (t, 3H, J = 7 Hz, C3:-$CH_2CH_2CH_3$), 1.2–1.65 (m, 4H, C3:-$CH_2CH_2CH_3$), 2.1–2.55 (m, 8H, N1:-CH_2-CH_2-), 3.45 (m, 4H, N1:-CH_2-CH_2-), 4.58 (m, 1H, C3:-*H*), 7.25–7.65 (m, 8 arom. H); ^{13}C NMR ($CDCl_3$) δ 14.1 (C3:-$CH_2CH_2CH_3$), 19.3 (C3:-$CH_2CH_2CH_3$), 33.5, 33.9 (C3:-$CH_2CH_2CH_3$), 43.2, 53.9, 55.2, 63.3, (C-3), 66.8, 123.4, 128.2, 129.6, 130.4, 131.2, 136.5, 140.8, 166.3, 169.3 ppm.

*7-Chloro-1-(3,3-dimethyl-2-oxobutyl)-5-phenyl-3-propyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (**6s**)*

APCI+m/s: m/z = 411 (90%), 313 (5%), 268 (5%); TLC (ether): R_f = 0.72; IR (KBr) ν_{max} = 2957, 2875, 1717, 1675, 1598, 1482, 1283, 1121, 1069, 736, 690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.8–1.05 (m, 12H, C3:-$CH_2CH_2CH_3$+N1:-CH_2-COC(CH_3)$_3$, 1.4–1.9 (m, 4H, C3:-$CH_2CH_2CH_3$), 4.25 (s, 2H, N1:-CH_2-COC(CH_3)$_3$), 4.7 (tr, 1H, J = 5 Hz, C-3), 7.0–7.75 (m, 8 arom. H); ^{13}C NMR ($CDCl_3$) δ 13.9 (C3:-$CH_2CH_2CH_3$), 19.6 (C3:-$CH_2CH_2CH_3$), 26.4 (N1:-CH_2-COC(CH_3)$_3$), 33.5 (C3:-$CH_2CH_2CH_3$), 53.3 (N1:-CH_2-COC(CH_3)$_3$), 63.8 (C3:-H), 122.3, 128.2, 128.9, 129.6, 130.5, 131.2, 136.8, 141.7, 167.2, 169.3, 209.4 (N1:-CH_2-COC(CH_3)$_3$) ppm.

*2-(7-Chloro-2-oxo-5-phenyl-3-propyl-2,3-dihydro-1*H*-1,4-benzodiazepin-1-yl)acetamide (**6u**)*

APCI+m/s: m/z = 370 (90%), 313 (5%), 279 (5%); TLC (ether): R_f = 0.52; IR (KBr) ν_{max} = 3405, 2955, 2869, 1723, 1663, 1463, 1278, 1128, 1069, 740, 690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.99 (t, 3H, J = 7 Hz, C3:-$CH_2CH_2CH_3$), 1.3–1.8 (m, 4H, C3:-$CH_2CH_2CH_3$), 4.2 (s, 2H, N1:-CH_2-CO-NH_2), 4.55 (m, 1H, C3:-H), 5.82+6.45 (2 s, 2H, N1:-CH_2-CONH_2), 7.25–7.7 (m, 8 arom. H); ^{13}C NMR ($CDCl_3$) δ 13.9 (C3:-$CH_2CH_2CH_3$), 22.9 (C3:-$CH_2CH_2CH_3$), 28.9 (C3:-$CH_2CH_2CH_3$), 57.1 (N1:-CH_2-CO-NH_2), 65.3 (C3:-H), 126.4, 128.2, 128.6, 129.5, 130.4, 130.8, 131.2, 131.6, 135.0, 139.4, 141.2, 167.7 (C═O), 170.6, 192.3 (N1:-CH_2-CO-NH_2) ppm.

Pharmacology

^{131}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-365,260 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 rev min^{-1}. It was then centrifuged at 1000 *g* (3000 rev min^{-1}) for 10 min. The supernatant was centrifuged at 20000 *g* (13000 rev min^{-1}) for 20 min. This pellet was redispensed in the required volume of assay buffer as defined below with 5 strokes of homogenizer at 500 rev min^{-1}. The final tissue concentration was 1 g original weight/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 (25 pM) to be tested were incubated 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.

The GABA-A binding assay was performed as described by Speth et al (1979) using ^3H-diazepam.

In-vivo evaluation: black and white box test, elevated x-maze and motor activity

Male OF1 mice (20–25 g) were kept in conventional plastic cages in groups of 10 and had free access to food and water. They were housed in air-conditioned facilities at 27–28°C on a 12-h light–dark cycle. The animal work was approved by the Bioethics committee of Khon Kaen University Faculty of Medicine (HO 2434-76).

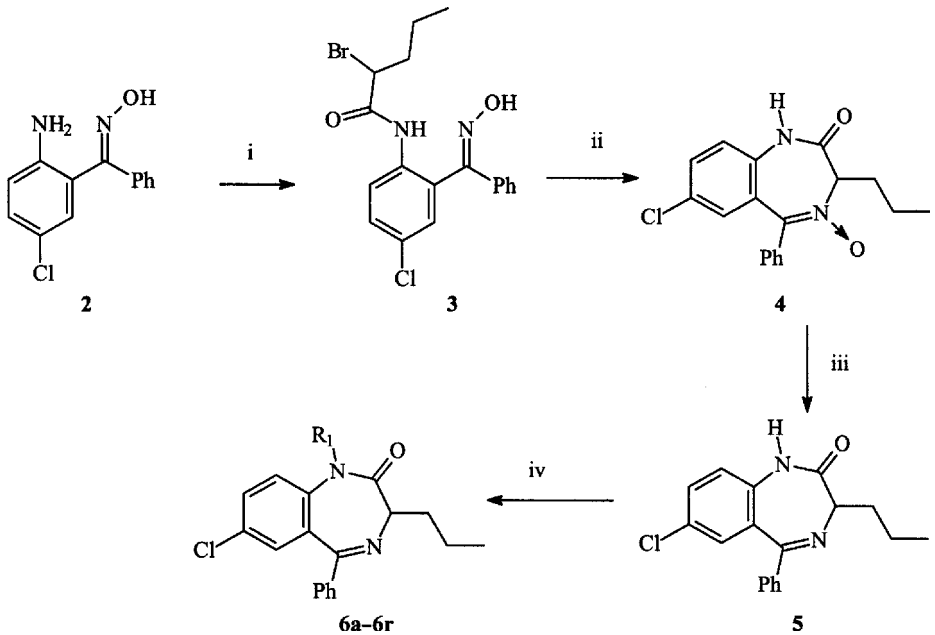


Figure 2 Synthesis of N-alkylated 3-propyl-1,4-benzodiazepines. Reagents: i. Bromovaleryl-bromide, reflux, dichloroethane; ii. KOH, pH 11, RT; iii. PCl_3, $CHCl_3$, reflux; iv. NaH, THF, RT or 50°C, 1.2 eq E^+ (Table 1).

The test compounds and diazepam (as standard) were dissolved in propylene glycol. The solution was administered intraperitoneally at a volume of 0.05 mL/10 g at 30 min before the experiments; the group size was 7 for each dose. The time the mice spent in the open arm was recorded for an observation period of 5 min in the elevated x-maze test. The time spent in the light was recorded for an observation period of 10 min in the black and white box test. The equipment was purchased from San Diego Instruments (7758 San Diego, CA 92126). The motor activity of the mice was recorded as the time that the mice were able to hold on the sieve at an angle of 180 °. The data were tested by using one-way analysis of variance (Tukey test) and the full experimental details of each assay are available from Vogel & Vogel (1997).

Results and Discussion

Chemistry

A synthetic approach towards the synthesis of the 3-propyl-1,4-benzodiazepine **5** and its alkylation in the N_1-position are outlined in Figure 2.

2-Amino-4-chloro-amino-benzophenone (Sternbach et al 1962) was converted into the corresponding oxime **2** with hydroxylamine, which was subsequently reacted with bromo-butyric acid chloride giving the desired amide **3** in high yield. Under basic conditions, the cyclization furnished the N-oxide **4**, unlike the known Polonowski rearrangement (Bell & Childress 1962). Compared with the hydroxy group, the more nucleophilic nitrogen reacted in the 7-exo-tet reaction only into the desired N-oxide **4**. No formation of the 3-hydroxy-3-propyl-1,4-benzodiazepine (oxazepam analogue) via an 8-membered ring was observed. The N-oxide **4** was subsequently reduced to the target **5** by using an excess of phosphorous trichloride.

The N-alkylated benzodiazepines **6a–6r** were synthesized from the 1,4-benzodiazepine template **5** with sodium hydride in tetrahydrofuran at ambient temperature or 50°C (Evans et al 1987). No dialkylation products were obtained and no column chromatography was required for further purification.

Pharmacology and structure–activity relationship studies

The ligand containing a C3 unit in the 3-position of the 1,4-benzodiazepine was previously found to bind best to a postulated lipophilic pocket at the CCK_2 receptor (Lattmann et al 2001). In order to optimize the affi nity of the 3-propyl-1,4-benzodiazepines, additional substituents had to be introduced in the N_1-position, as are outlined in Table 1.

More complex electrophiles forming piperidino-, morpholino- and phthalimido-benzodiazepines **6o–6q**

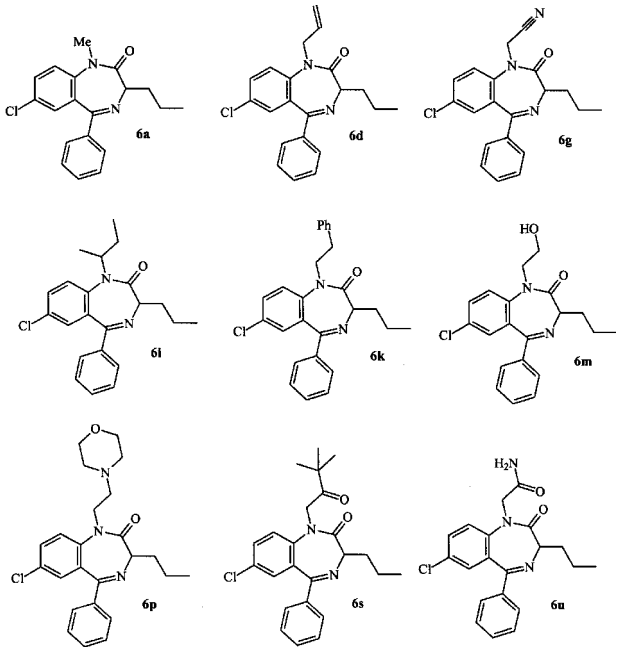


Figure 3 Overview of selected 1,4-benzodiazepines.

did not show an enhanced binding affinity on the CCK receptor. As previously reported for the 3-ureido-benzodiazepines, N_1-alkylation (Semple et al 1996) should have shown enhanced binding on the CCK_2 receptor, but with the majority of the electrophiles used here the affinity was significantly lower than for the N_1-unalkylated 1,4-benzodiazepines.

Functionalized hydrophilic substituents such as the hydroxy-alkyl series **6l**–**6m** resulted in the loss of binding.

In order to gain a high chemical diversity, an amide **6u**, an ester **6r** and two ketones **6s** and **6t** were attached to the N_1-position. Unlike the affinity observed for the N_1 alkylated propyl-1,4-benzodiazepines **6s** and **6t**, Merck's 3-ureido-1,4-benzodiazepines have shown the best binding with the N_1-substituents, analogues to the compounds **6s** and **6t** (Semple et al 1997).

The benzodiazepines **6d**–**6 g**, containing a cyanomethyl-, propargyl- and allyl-group, displayed an increased affinity. Obviously, large and bulky substituents reduce the binding affinity, and small, lipophilic electron-rich groups, such as the allyl group, are ideal for an enhancement of the bioactivity. A substituent in the N_1 position is desirable, firstly to enhance the affinity and secondly to differentiate the binding profile between the CCK and the benzodiazepine receptor. It is known that most of the substituents in the N_1 position block the affinity on the GABA-A receptor (Martin & Lattmann 1999) (Figure 3).

In-vivo studies

The neuropharmacological effects of the 3-propyl benzodiazepine template **5** and the best 1,3-dialkylated benzodiazepine **6d** were evaluated in comparison with diazepam in four different in-vivo assays in mice. The cyanomethyl benzodiazepine **6 g**, with a similar affinity to **6d**, was excluded from further evaluation owing to insolubility in water and organic solvents. Compounds **5** and **6d** have been found to be inactive in the evaluation of pain in the hot-plate and the tail-flick assays (O'Neill et al 1989).

The anxiolytic effect was evaluated by using the black and white box test (Matto et al 1997) and the elevated x-maze (Johnson & Rodgers 1996) as two standard anxiolytic assays. For diazepam, the anxiolytic activity correlated with sedation/muscle relaxation. The 3-propyl-benzodiazepine **5** displayed a week anxiolytic effect, having an ED50 of approximately 20 mg kg^{-1}. In contrast to **5**, the dialkylated 1,4-benzodiazepine **6d** displayed an unexpectedly strong anxiolytic effect, with a magnitude comparable with diazepam (Table 2). Based on doses of 1, 5, 20, 100 and 200 mg kg^{-1}, the ED50 values for the black and white box and x-maze were 4.7 and 5.2 mg kg^{-1}, respectively, with no reduced motor activity observed. A significant sedation/muscle relaxation compared with the control was observed at 90 mg kg^{-1} (ED50), a dose that is 18-times greater than

Table 2 Results of receptor binding data compared with in-vivo studies (elevated x-maze, black and white box model and motor activity).

	Receptor binding	**ED50 (mg kg^{-1})**		
		Elevated x-maze	Black and white box	Motor activity
Diazepam	GABA-A: 70±5 nM CCK_2: >100±11 μM	1.1±0.2	1.3±0.3	1.3±0.2
6d	GABA-A: >50±7 μM CCK_2:170±23 nM	5.2±0.9	4.7±0.8	>200±15

the effective anxiolytic dose. In a literature search, a similar anxiolytic compound, dihydrohonokiol, with a propyl and an allyl group attached to an aromatic centre, was found to act on the steroid-binding site of the GABA receptor (Stavinoha 1999).

Conclusion

A novel five-step chemical approach from 2-aminobenzophenone towards the synthesis of 3-*n*-propyl-1,4-benzodiazepines was developed. Introducing substituents in the N_1-position further enhanced the affinity of the 1,4-benzodiazepine template. Based on the results of the receptor-binding assay, the best compounds were evaluated in animal experiments. The 1-allyl-3-propyl-1,4-benzodiazepine **6d**, having an IC50 of approximately 0.17 μM, was found to be active as a novel anxiolytic with an ED50 of approximately 5 mg kg^{-1} in the x-maze and the black and white box assays. There is a current development programme to optimize this lead structure and to study the binding profile of these novel anxiolytics.

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