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Synthesis and Biological Evaluation of New 3-Aralkylamino-2-aryl-2*H*-1,2,4-pyridothiadiazine 1,1-dioxides as Potential CCK-Receptor Ligands

PASCAL DE TULLIO, BERNARD PIROTTE, PHILIPPE NEVEN, BERNARD MASEREEL, DANIEL DEWALQUE, OUSMANE DIOUF, TCHAO PODONA, DANIEL CAIGNARD*, PIERRE RENARD* AND JACQUES DELARGE

*Department of Medicinal Chemistry, University of Liège, 3 rue Fusch, B-4000 Liège, Belgium and *Adir & Cie, 1 rue Carle Hebert, F-92415 Courbevoie, France*

Abstract

A series of 2-aralkyl-4*H*-pyridothiadiazine 1,1-dioxides and 3-aralkylamino-2-aryl-2*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides structurally related to quinazolinone CCK receptor antagonists were synthesized and evaluated as CCK-A and CCK-B receptor ligands.

The compounds were effective as cholecystokinin-ligands in the micromolar range of concentration, c.f. the cholecystokinin receptor antagonists asperlicin, lorglumide or benzotript, and were thus less potent than the best quinazolinones previously reported.

Although the compounds were unsuitable for drug use, the work contributed to our understanding of the chemistry of unusual 2,3-disubstituted pyridothiadiazinedioxides.

During the last few years cholecystokinin (CCK) has emerged as an important polypeptide hormone (Crawley & Corwin 1994). This neuropeptide has been located in peripheral tissues such as the duodenum, jejunum, pancreas and gut (Baile et al 1986) and in the central nervous system (Cherner et al 1988). The primary structure and the biological properties of cholecystokinin are closely related to those of gastrin, another intestinal peptide. Because cholecystokinin is involved in many different biological processes such as gut function, digestive processes, control of feeding behaviour and neurotransmitter release, the therapeutic potential of cholecystokinin receptor ligands, and in particular of antagonists, seemed to be extremely broad and promising (Iversen et al 1991; Wettstein et al 1994). Such compounds are expected to inhibit cholecystokinin-stimulated gall-bladder contraction (Liddle et al 1989) and pancreatic exocrine secretion (Rehfeld et al 1980; Jensen et al 1989); they could also resolve many problems associated with gastric motility (Hersey et al 1983); they act on pain treatment (McRoberts 1986), appetite control (Blundell 1991), anxiety and panic crisis (Singh et al 1991). Cholecystokinin receptors are divided into at least two categories (Moran et al 1986; Yu et al 1990): the CCK-A receptor (A for 'alimentary') essentially located in the peripheral tissues, and the CCK-B receptor (B for 'brain') mainly found in the central nervous system (Woodruff & Hughes 1991).

The first cholecystokinin receptor ligands to be developed were based on studies of cholecystokinin primary structure and were peptide or pseudopeptide derivatives (Freidinger 1989; Nadzan & Kerwin 1991). The discovery of the first potent non-peptide antagonist asperlicin (Fig. 1) completely changed the design of cholecystokinin receptor antagonists (Chang et al 1985). Asperlicin is a natural product isolated from the fungus *Aspergillus alliaceus* (Goëtz et al 1985). From a chemical point of view, this drug integrates three different heterocyclic systems: a benzodiazepine, a quinazolinone and an indole ring. Simplification of the structure of this complex molecule provided two principal directions in cholecystokinin ligand research. The first focused on the benzodiazepine ring system and led to very potent and selective CCK-A receptor antagonists (Evans et al 1987) such as devazepide (Louie et al 1988) but also of CCK-B receptor antagonists such as L-740,093 (Patel et al 1994; Fig. 1). The second direction was based on the quinazolinone heterocyclic core structure (Yu et al 1991). This approach led to potent CCK-B receptor antagonists such as I (Fig. 1; Yu et al 1992). It was recently reported that the chronic therapeutic use of CCK-A antagonists, especially the benzodiazepine-type compounds, was compromised. In contrast, interest in CCK-B receptor antagonists for the treatment of anorexia, panic and anxiety states or for the enhancement of the effectiveness of opiate analgesics, still remained important (Patel et al 1994; Satoh et al 1995). It was noted that most non-peptide CCK-B receptor antagonists also had strong antagonistic activity on the gastrin receptors.

The bioisostery between the 3,4-dihydroquinazolin-4-ones and the 1,2,4-benzothiadiazine 1,1-dioxides is now a well established concept (i.e. the thiazide and quinazolinone diuretics; Pirotte et al 1990). We have, moreover, confirmed the structural similarity between benzo- and pyridothiadiazine dioxides in the field of potassium channel openers (de Tullio et al 1995, 1996). We therefore decided to prepare and to test novel 3-aralkyl-2-aryl-2*H*-1,2,4-pyridothiadiazine 1,1-dioxides as original cholecystokinin receptor ligands. As shown in Scheme 1, this kind of compound appeared to be strongly related to the powerful quinazolinone CCK-B receptor antagonists.

In order to appreciate the importance of the pyridinic nitrogen atom position on biological activity, we first synthe-

Correspondence: P. de Tullio, Department of Medicinal Chemistry, University of Liège, 3 rue Fusch, B-4000 Liège, Belgium.

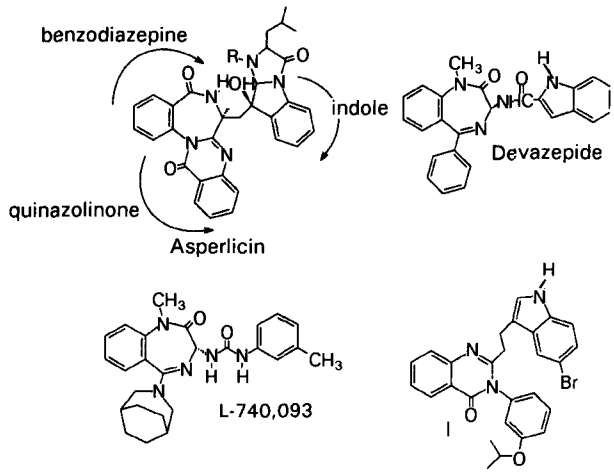
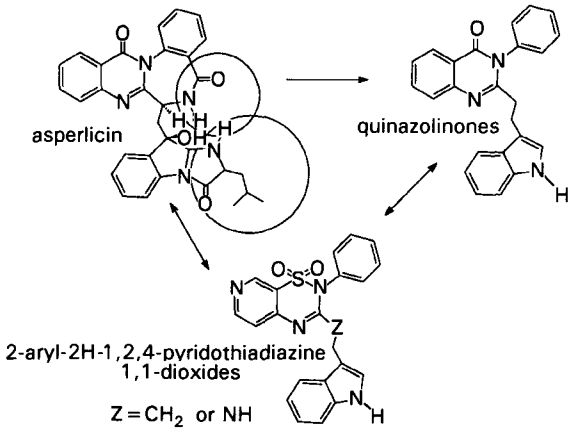


FIG. 1. Asperlicin and its principal non-peptide CCK receptor ligand derivatives.



SCHEME 1. Structural analogues among asperlicin, quinazolinones and pyridothiadiazinedioxides.

sized three 2-unsubstituted pyridothiadiazine dioxides 7 to 9. Because no suitable data were available about the best pyridinic nitrogen position, we chose the pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide ring system for reasons of accessibility.

Because 3-aralkyl-2-aryl-2*H*-1,2,4-benzo- and pyridothiadiazine 1,1-dioxides were, moreover, not available, we decided to prepare chemically accessible 3-aralkylamino analogues. In all cases, 3-aralkyl or 3-aralkylamino groups were related to the indole group contained by the quinazolinone antagonists. We also modified the linker lengths connecting the two heteroaromatic domains: the indole and the pyridothiadiazine rings, and also replaced the indole system by a naphthalene one commonly expected as bioisostere of the forgoing one. We provided a phenyl ring in place of the indole nucleus in order to verify the importance of the indole nucleus in receptor binding. Finally, in consideration of reported quinazolinone binding results, we introduced an electronegative region on the 3-aralkylamino side chain (Yu et al 1992). The choice of the substituent on the aryl group in the 2-position of the thiadiazine ring was also based on structure activity relationships noted for quinazolinone derivatives (Yu et al 1991).

Materials and Methods

Chemistry

Melting points were determined on a Büchi-Totolli capillary apparatus and are uncorrected. IR spectra were recorded as KBr pellets on a Perkin-Elmer 1750 FT spectrophotometer. ^{1}H NMR spectra were obtained on a Bruker AW-80 (80 MHz) instrument in DMSO-d_6 with hexamethyldisiloxane (HMDS) as internal standard; chemical shifts are reported as δ values (ppm) relative to internal HMDS. The abbreviations s = singlet, d = doublet, t = triplet, m = multiplet and b = broad are used throughout. Elemental analysis (C, H, N, S) were performed with a Carlo-Erba EA 1108-elemental analyser and were within ±0·4% of theoretical values. All reactions were routinely checked by TLC on Merck silica gel 60F_{254}.

Succinimidyl 3-(1H-indol-3-yl)propionate. 3-(1*H*-Indol-3-yl)-propionic acid (10 g, 0·053 mol) and *N*-hydroxysuccinimide (6 g, 0·058 mol) were dissolved in THF (100 mL). A solution of dicyclohexylcarbodiimide (12 g, 0·058 mol) in THF (30 mL) was added dropwise. After stirring for 5 h at room temperature the precipitated dicyclohexylurea was removed by filtration. The filtrate was concentrated under reduced pressure and the residue was dissolved in ethyl acetate (300 mL) and left to stand at +4°C for 12 h. The insoluble material formed was removed by filtration. The organic layer was washed with $NaHCO_3$ solution (4%; 600 mL) and with water (300 mL). After drying over $MgSO_4$, petroleum ether (bp 40–60°C; 500 mL) was added to the ethyl acetate solution. The resulting precipitate was collected by filtration, washed with petroleum ether (bp 40–60°C) and dried (yield 56%). mp 150°C (dec.); IR (KBr) 3368 (N–H), 1724, 1246 (C = O) cm^{-1}; ^{1}H NMR δ 2·7 (s, 4H, indol-CH_2-CH_2-CO), 3·0 (s, 4H, COON-CO-CH_2-CH_2-CO), 6·6–7·8 (bm, 5H, *indole*), 10·8 (bs, 1H, N*H* indole). Anal. ($C_{15}H_{14}N_2O_4$) C, H, N.

N-(3-Aminopyridine-2-sulphonyl)-3-(1H-indol-3-yl)propanamide (4). 3-Aminopyridine-2-sulphonamide sodium salt, **1** (0·34 g, 1·96 mmol) was dissolved in DMF (4 mL). A solution of succinimidyl 3-(1*H*-indol-3-yl)propionate (0·56 g, 1·96 mmol) in dioxane (4 mL) was added dropwise to the sodium salt. After stirring for 1 h at 60°C, most of the solvent was removed by distillation under reduced pressure. The residue was dissolved in an aqueous solution of NaOH (10%; 25 mL). The solution was treated with charcoal and filtered. The filtrate was adjusted to pH 5 with formic acid and the precipitate was collected by filtration, washed with water and dried (yield 65%). mp 211–213°C; IR (KBr) 3479, 3426, 3370 (N-H), 1687 (C = O), 1618, 1593, 1524 (C = C, C = N, N-H), 1333, 1145 (S = O) cm^{-1}; ^{1}H NMR δ 2·4 (t, 2H, CO-CH_2CH_2-indole), 2·7 (t, 2H, CO-CH_2CH_2-indole), 3·2 (bs, SO_2-N*H*-CO + H_2O), 6·1 (bs, 2H, 3-NH_2), 6·8-7·5 (bm, 7H, 4-*H* + 6-*H* + *indole*), 7·75 (m, 1H, 5-*H*), 10·6 (bs, 1H, N*H* indole). Anal. $C_{16}H_{16}N_4O_3S$) C, H, N, S.

N-(4-Aminopyridine-3-sulphonyl)-3-(1H-indol-3-yl)propanamide (5). This compound was obtained from 4-aminopyridine-3-sulphonamide sodium salt, **2**, by the procedure used to obtain 4 (yield 70%). mp 198–201°C; IR (KBr) 3393, 3312, 3229 (N–H), 1656 (C = O), 1632, 1587, 1533, 1519 (C = C, C = N,

N-H), 1284, 1153 (S=O) cm^{-1}; ^1H NMR δ 2·5 (t, 2H, CO-CH_2CH_2-indole), 2·8 (bt, 2H, CO-CH_2CH_2-indole), 3·2 (bs, SO_2-NH-CO + H_2O), 6·8 (d, 1H, 5-*H*), 6·9-7·6 (bm, 7H, *indole* + NH_2), 8·1 (d, 1H, 6-*H*), 8·5 (s, 1H, 8-*H*), 10·7 (bs, 1H, N*H* indole). Anal. ($C_{16}H_{16}N_4O_3S$) C, H, N, S.

N-*(2-Aminopyridine-3-sulphonyl)-3-(1*H*-indol-3-yl)propanamide (**6**).* This compound was obtained from 2-aminopyridine-3-sulphonamide sodium salt, 3, by the procedure used to obtain **4** (yield 75%). mp 117-121°C; IR (KBr) 3495, 3372, 3241 (N-H), 1707 (C=O), 1658, 1615, 1589, 1558 (C=C, C=N, N-H), 1326, 1174 (S=O) cm^{-1}; ^1H NMR δ 2·5 (t, 2H, CO-CH_2CH_2-indole), 2·75 (bt, 2H, CO-CH_2CH_2-indole), 6·4-7·5 (bm, 8H, $2NH_2$ + *indole* + 5-*H*), 7·9 (bd, 1H, 4-*H*), 8·15 (bd, 1H, 6-*H*), 10·7 (bs, 1H, N*H* indole), 12·0 (bs, SO_2NHCO). Anal. ($C_{16}H_{16}N_4O_3S$) C, H, N, S.

*3-[2-(1*H*-Indol-3-ylethyl)]-4*H*-pyrido[3,2-e]-1,2,4-thiadiazine 1,1-dioxide (7).* *N*-(3-Aminopyridine-2-sulphonyl-3-(1*H*-indol-3-yl)propanamide, **4** (0·9 g, 2·6 mmol) and pyridinium *p*-toluenesulphonate (0·26 g, 1·05 mmol) were dissolved in pyridine (15 mL). The mixture was heated under reflux for 6 h, cooled, and the solvent was removed by distillation under reduced pressure. The residue was suspended in water (10 mL). The resulting solid was triturated with diethyl ether and collected by filtration. The precipitate was dissolved in a 9:1 mixture of $CHCl_3$ and MeOH, treated with charcoal and filtered. The filtrate was concentrated under reduced pressure and the residue was dissolved in a dilute aqueous solution of NaOH (10 mL). The solution was adjusted to pH 5 with formic acid and the resulting precipitate of compound **7** was collected by filtration, washed with water and dried (yield 30%). mp 262–264 °C; IR (KBr) 3253, 3181 (N–H), 1615, 1571, 1530 (C=C, C=N, N-H), 1289, 1169 (S=O) cm^{-1}; ^1H NMR δ 3·0 (m, 4H, C*H*2-CH_2), 6·9–7·65 (m, 7H, 5-*H* + 7-*H* + indole), 8·5 (m, 1H, 6-*H*), 10·7 (bs, 1H, N*H*-indole), 12·1 (bs, 1H, 4-N*H*). Anal. ($C_{16}H_{14}N_4O_2S$) C, H, N, S.

*3-[2-(1*H*-Indol-3-ylethyl)]-4*H*-pyrido[4,3-e]-1,2,4-thiadiazine 1,1-dioxide (**8**).* *N*-(4-Aminopyridine-3-sulphonyl)-3-(1*H*-indol-3-yl)propanamide 5 (0·4 g, 1·16 mmol) was fused at 200°C for 1 h. After cooling, crystallization of the crude residue from 1:3 MeOH-diethyl ether provided 8 as a white solid (yield 55%). mp 257-260°C; IR (KBr) 3472, 3439, 3353, 3268 (N-H), 1628, 1611, 1572, 1506 (C=C, C=N, N-H), 1270, 1158 (S=O) cm^{-1}; ^1H NMR δ 2·95 (m, 4H, 3-CH_2-CH_2), 6·8-7·6 (m, 6H, 5-*H* + *indole*), 8·4 (s, 1H, 6-*H*), 8·9 (s, 1H, 8-*H*), 10·7 (bs, 1H, N*H*-indole), 12·1 (bs, 1H, 4-NH). Anal. ($C_{16}H_{14}N_4O_2S$) C, H, N, S.

*3-[2-(1*H*-Indol-3-ylethyl)]-4*H*-pyrido[2,3-e]-1,2,4-thiadiazine 1,1-dioxide (**9**).* *N*-(2-Aminopyridine-3-sulphonyl)-3-(1*H*-indol-3-yl)propanamide **6** (2 g, 5·8 mmol) was fused at 180°C for 30 min. After cooling, the solid residue was dissolved in aqueous NaOH (10%; 30 mL), treated with charcoal and filtered. The filtrate was adjusted to pH 4 with formic acid and the precipitate of **9** was collected by filtration, washed with water and dried (yield 78%). mp 241-244°C; IR (KBr) 3344, 3235 (N–H), 1661, 1613, 1574, 1520 (C=C, C=N, N-H), 1290, 1139 (S=O) cm^{-1}; ^1H NMR δ 3·0 (m, 4H, 3-CH_2-CH_2), 6·8–7·6 (m, 6H, 7-*H* + *indole*), 8·25 (d, 1H, 6-*H*), 8·55 (d, 1H, 8-*H*), 10·7 (s, 1H, N*H*-indole), 12·6 (bs, 1H, 4-N*H*). Anal. ($C_{16}H_{14}N_4O_2S$) C, H, N, S.

N-*Phenyl-4-chloropyridine-3-sulphonamide (11).* This compound was obtained from 4-chloropyridine-3-sulphonyl chloride, **10**, and aniline by the procedure used to obtain 12 (yield 50%). mp 178–180°C; IR (KBr) 3425 (N-H), 1644, 1597, 1569, 1545 (C=C, C=N, N-H), 1350, 1167 (S=O) cm^{-1}; ^1H NMR δ 7·1 (bs, 5H, phenyl), 7·65 (d, 1H, 5-*H*), 8·65 (d, 1H, 6-*H*), 8·95 (s, 1H, 8-*H*), 10·8 (bs, 1H, SO_2N-*H*). Anal. ($C_{11}H_9N_2O_2SCl$) C, H, N, S.

N-*(3-Isopropoxyphenyl)-4-chloropyridine-3-sulphonamide (**12**).* Crude 4-chloropyridine-3-sulphonyl chloride, **10** (0·099 mol) was dissolved in dioxane (100 mL) and added dropwise to a solution of 3-isopropoxyaniline (9 g, 0·059 mol) and triethylamine (18 mL) in dioxane (50 mL). After stirring for 1 h at room temperature the solvent was removed by distillation under reduced pressure. The residue was dissolved in a dilute aqueous solution of NaOH and the residual precipitate was removed by filtration. The filtrate was adjusted to pH 4 with 1 M HCl and the final product was collected by filtration, washed with water and dried (yield 46%). mp 127–129°C; IR (KBr) 3425 (N–H), 1596, 1566, 1550, 1506 (C=C, C=N,N-H), 1347, 1189 (S=O) cm^{-1}; ^1H NMR δ 1·1 (d, 6H, O-CH(CH_3)$_2$), 4·35 (m, 1H, O-C*H*(CH_3)$_2$), 6·55 (m, 3H, 2′-*H* + 4′-*H* + 6′-*H* phenyl), 7·05 (m, 1H, 5′-*H* phenyl), 7·7 (d, 1H, 5-*H*), 8·65 (d, 1H, 6-H), 8·95 (s, 1H, 8-*H*), 10·8 (bs, 1H, SO_2N-*H*). Anal. ($C_{14}H_{15}N_2O_3SCl$) C, H, N, S.

N-*Phenyl-4-aminopyridine-3-sulphonamide (13).* This compound was obtained from *N*-phenyl-4-chloropyridine-3-sulphonamide, **11**, by the procedure used to obtain **14** (yield 86%). mp 241–243°C; IR (KBr) 3448, 3357 (N-H), 1641, 1600, 1544 (C=C, C=N, N-H), 1303, 1158 (S=O) cm^{-1}; ^1H NMR δ 6·6 (bs, 2H, 4-NH_2), 6·7 (d, 1H, 5-*H*), 6·9-7·3 (bs, 5H, phenyl), 7·95 (d, 1H, 6-*H*), 8·3 (s, 1H, 8-*H*), 10·2 (bs, 1H, SO_2N-*H*). Anal. ($C_{11}H_{11}N_3O_2S$) C, H, N, S.

N-*(3-Isopropoxyphenyl)-4-aminopyridine-3-sulphonamide (14).* A suspension of *N*-(3-isopropoxyphenyl)-4-chloropyridine-3-sulphonamide, **12** (2·5 g, 7·6 mmol) in concentrated ammonia (25 mL) was heated in a hermetically closed autoclave at 150°C for 12 h. After cooling the reaction mixture was concentrated to small volume (10 mL) under reduced pressure to give the white crystalline product, **14**, which was collected by filtration, washed with water and dried (yield 85%). mp 214–216°C; IR (KBr) 3453, 3359 (N-H), 1640, 1597, 1600, 1544, 1505 (C=C, C=N, N-H), 1321, 1161 (S=O) cm^{-1}; ^1H NMR δ 1·1 (d, 6H, O-CH(CH_3)$_2$), 4·45 (m, 1H, O-C*H*(CH_3)$_2$), 6·4-7·3 (bm, 7H, 4-NH_2 + *phenyl* + 5-*H*), 7·95 (d, 1H, 6-*H*), 8·35 (s, 1H, 8-*H*), 10·2 (bs, 1H, SO_2N-*H*) Anal. ($C_{14}H_{17}N_3O_3S$) C, H, N, S.

*3-(1*H*-Imidazol-1-yl)-2-phenyl-2*H*-pyrido[4,3-e]-1,2,4-thiadiazine 1,1-dioxide (**16**).* This compound was obtained from *N*-phenyl-4-aminopyridine-3-sulphonamide, 13, by the procedure used to obtain **17** (54%). mp 213–215°C; IR (KBr) 1616, 1592, 1574, 1543, 1525 (C=C, C=N, N-H), 1312, 1173 (S=O) cm^{-1}; ^1H NMR δ 6·9 (m, 1H, 4-*H* imidazole), 7·35 (bs, 5H, *phenyl*), 7·55 (bs, 1H, 5-*H* imidazole), 7·75 (d, 1H, 5-

H), 8·3 (s, 1H, 2-*H* imidazole), 9·0 (d, 1H, 6-*H*), 9·1 (s, 1H, 8-*H*). Anal. ($C_{15}H_{11}N_5O_2S$) C, H, N, S.

3-(1H-Imidazol-1-yl)-2-(3-isopropoxyphenyl)-2H-pyrido[4,3-e]-1,2,4-thiadiazine 1,1-dioxide (**17**). 1,1′-thiocarbonyldiimidazole (5·35 g, 30 mmol) was added to a solution of *N*-(3-isopropoxyphenyl)-4-aminopyridine-3-sulphonamide **14** (3 g, 9·8 mmol) in dioxane (40 mL) and DMF (10 mL). After stirring under reflux for 90 min the solvent was removed by distillation under reduced pressure and the residue was dissolved in water (30 mL). The mixture was adjusted to pH 12 with a dilute aqueous solution of NaOH (1 M). After cooling on ice, the resulting solid was collected and stirred in water (30 mL) for 12 h. The precipitate was filtered, dissolved in the minimum volume of MeOH and added to water until precipitation was complete. Recrystallization from $CHCl_3$-petroleum ether (bp 40–60°C) furnished **17** as a white solid (yield 48%). mp 145–147°C; IR (KBr) 1613, 1574, 1528 (C=C, C=N, N-H), 1319, 1186 (S=O) cm^{-1}; ^1H NMR δ 1·15 (d, 6H, O-CH(CH_3)$_2$), 4·55 (m, 1H, O-C*H*(CH_3)$_2$), 6·8–7·3 (m, 5H, *phenyl* + 4-*H* imidazole), 7·6 (s, 1H, 5-*H* imidazole), 7·75 (d, 1H, 5-*H*), 8·3 (s, 1H, 2-*H* imidazole), 8·95 (d, 1H, 6-*H*), 9·1 (s, 1H, 8-*H*). Anal. ($C_{18}H_{17}N_5O_3S$) C, H, N, S.

3-Aminomethyl-1H-indole hydrochloride (**20**). A solution of (1*H*-indol-3-yl)carbonitrile (4 g, 28 mmol) in anhydrous diethyl ether (200 mL) was added dropwise to a suspension of $LiAlH_4$ (3·2 g, 84·4 mmol) in anhydrous diethyl ether (50 mL). After stirring for 1 h at room temperature, water (400 mL) was added slowly to the mixture and the solution was extracted with ethyl acetate (1 L). The organic layer was dried over $MgSO_4$ and concentrated to small volume (300 mL) under reduced pressure. This organic phase was extracted with dilute HCl (400 mL) and the aqueous layer was then treated with charcoal and concentrated under reduced pressure. The residue was dissolved in the minimum volume of ethanol and diethyl ether was added until complete precipitation (28%). mp 85-87°C; ^1H NMR δ 4·1 (bt, 2H, NH_2-C*H*$_2$-indole), 6·8–7·8 (bm, 5H, *indole*), 8·2 (bs, 3H, NH_3+), 11·3 (bs, 1H, N*H* indole). Anal. ($C_9H_{11}N_2O_2Cl$) C, H, N.

3-Benzylamino-2-phenyl-2H-pyrido[4,3-e]-1,2,4-thiadiazine 1,1-dioxide (**21**). A solution of 3-(1*H*-imidazol-1-yl)-2-phenyl-2*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide, **16** (0·4 g, 1·23 mmol) and benzylamine (0·4 mL) in dioxane (4 mL) was heated under reflux for 30 min. The solvent was removed by distillation under reduced pressure and the residue was suspended in water. The mixture was adjusted to pH 4 with formic acid and the resulting precipitate was collected by filtration, washed with water and dried. Recrystallization from $CHCl_3$-petroleum ether (bp 40–60°C) furnished **21** (yield 87%). mp 164–167°C; IR (KBr) 3423 (N-H), 1613, 1548, 1496 (C=C, C=N, N-H), 1341, 1176 (S=O) cm^{-1}; ^1H NMR δ 4·5 (bd, 2H, 3-NH-C*H*$_2$-phenyl), 6·9-7·5 (bm, 11H, 5-*H* + 2-*phenyl* + 3-*phenyl*), 7·95 (bs, 1H, 3-N*H*), 8·55 (d, 1H, 6-*H*), 8·75 (s, 1H, 8-*H*). Anal. ($C_{19}H_{16}N_4O_2S$) C, H, N, S.

3-Benzylamino-2-(3-isopropoxyphenyl)-2H-pyrido[4,3-e]-1,2,-4-thiadiazine 1,1-dioxide (**22**). A suspension of 3-(1*H*-imidazol-1-yl)-2-(3-isopropoxyphenyl)-2*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide, 17 (0·4 g, 1·04 mmol) in benzylamine (0·4 mL) was heated for 1 h at 80°C. Excess amine was removed by distillation under reduced pressure and the residue was suspended in water. The mixture was adjusted to pH 4 with formic acid and the resulting precipitate was collected by filtration, washed with water and dried. Recrystallization from 1:3 MeOH-H_2O furnished **22** (yield 68%). mp 196–200°C; IR (KBr) 3423(N-H), 1614, 1598, 1587, 1547 (C=C, C=N, N-H), 1332 (S=O) cm^{-1}; ^1H NMR δ 1·2 (d, 6H, O-CH(C*H*$_3$)$_2$), 4·6 (m, 3H, 3-N*H*-C*H*$_2$-phenyl + O-C*H*(CH_3)$_2$), 6·75–7·55 (bm, 11H, 5-*H* + 2-*phenyl* + 3-*phenyl* + 3-N*H*), 8·55 (bd, 1H, 6-*H*), 8·75 (bs, 1H, 8-*H*). Anal. ($C_{22}H_{22}N_4O_3S$) C, H, N, S.

3-[(1H-Indol-3-ylmethyl)amino]-2-(3-isopropoxyphenyl)-2H-pyrido[4,3-e]-1,2,4-thiadiazine (**23**). A solution of 3-(1*H*-imidazol-1-yl)-2-(3-isopropoxyphenyl)-2*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide **17** (0·3 g, 0·78 mmol) and 3-aminomethyl-1*H*-indole freshly isolated from the hydrochloride, **21** (0·23 g, 1·56 mmol) in dioxane (0·5 mL) was heated under reflux under N_2 for 1 h. After removal of the solvent the mixture was suspended in water and adjusted to pH 4 with formic acid. The residual solid was collected by filtration, washed with water and dried. Recrystallization from 1:3 MeOH-H_2O furnished the product (yield 50%). mp 221–223°C; IR (KBr) 3402, 3210 (N-H), 1603, 1549, 1538, 1507 (C=C, C=N, N-H), 1330 (S=O) cm^{-1}; ^1H NMR δ 1·1 (d, 6H, O-CH(C*H*$_3$)$_2$), 4·35 (m, 1H, O-C*H*(CH_3)$_2$), 4·65 (d, 2H, 3-NH-C*H*$_2$-indole), 6·65–7·65 (m, 10H, 5-*H* + 2-*phenyl* + 3-*indole*), 7·85 (bs, 1H, 3-N*H*), 8·6 (d, 1H, 6-*H*), 8·75 (s, 1H, 8-*H*), 10·9 (s, 1H, N*H* indole). Anal. ($C_{24}H_{23}N_5O_3S$) C, H, N, S.

3-[(1H-Indol-3-ylethyl)amino]-2-(3-isopropoxyphenyl)-2H-pyrido[4,3-e]-1,2,4-thiadiazine (**24**). This compound was obtained from 3-(1*H*-imidazol-1-yl)-2-(3-isopropoxyphenyl)-2*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide, **17**, and tryptamine by the procedure used to obtain **23**. The compound was recrystallized from 1:3 $CHCl_3$-petroleum ether (bp 40–60°C) (yield 49%). mp 95-98°C; IR (KBr) 3414, 3186 (N-H), 1604, 1553, 1513 (C=C, C=N, N-H), 1339, 1175 (S=O) cm^{-1}; ^1H NMR δ 1·2 (d, 6H, O-CH(CH_3)$_2$), 2·9 (bt, 2H, 3-NHCH$_2$C*H*$_2$-indole), 3·55 (bt, 2H, 3-NHC*H*$_2$C*H*$_2$-indole), 4·5 (m, 1H, O-C*H*(CH_3)$_2$), 6·65 -7·7 (m, 11H, 5-*H* + 2-*phenyl* + 3-*indole* + 3-N*H*), 8·55 (d, 1H, 6-*H*), 8·75 (s, 1H, 8-*H*), 11·0 (s, 1H, N*H* indole). Anal. ($C_{25}H_{25}N_5O_3S$) C, H, N, S.

3-[(Naphth-1-ylmethyl)amino]-2-phenyl-2H-pyrido[4,3-e]-1,-2,4-thiadiazine 1,1-dioxide (**25**). This compound was obtained from 3-(1*H*-imidazol-1-yl)-2-phenyl-2*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide, **16**, and (naphth-1-yl)methylamine by the procedure used to obtain **21** (yield 84%). mp 222–224°C; IR (KBr) 3458 (N-H), 1608, 1598, 1538 (C=C, C=N, N-H), 1248, 1177 (S=O) cm^{-1}; ^1H NMR δ 4·95 (bd, 2H, 3-NH-C*H*$_2$-naphthyl), 7·1-8·3 (bm, 14H, 5-*H* + 2-*phenyl* + 3-N*H*-CH$_2$-*naphthyl*), 8·55 (d, 1H, 6-*H*), 8·75 (s, 1H, 8-*H*). Anal. ($C_{23}H_{18}N_4O_2S$) C, H, N, S.

3-[(Naphth-1-ylmethyl)amino]-2-(3-isopropoxyphenyl)-2H-pyrido[4,3-e]-1,2,4-thiadiazine 1,1-dioxide (**26**). A solution of 3-(1*H*-imidazol-1-yl)-2-(3-isopropoxyphenyl)-2*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide, **17** (0·5 g, 1·3 mmol) and (naphth-1-yl)methylamine (0·29 mL, 1·96 mmol) in dioxane (2·5 mL) was heated under reflux for 2 h. The mixture was

concentrated under reduced pressure and the residue was dissolved in water (50 mL). The solution was adjusted to pH 4 with formic acid and MeOH (7·5 mL) was added. The precipitate formed was collected by filtration, washed with water and dried (yield 81%). mp 192-194°C; IR (KBr) 3423 (N-H), 1603, 1584, 1538 (C=C, C=N, N-H), 1285, 1168 (S=O) cm^{-1}; ^1H NMR δ 1·15 (d, 6H, O-CH(CH_3)$_2$), 4·4 (m, 1H, O-C*H*(CH_3)$_2$), 5·0 (s, 2H, 3-NH-C*H*$_2$-naphthyl), 6·7–8·2 (bm, 13H, 5-*H* + 2-*phenyl* + 3-N*H*-CH_2-*naphthyl*), 8·5 (d, 1H, 6-*H*), 8·7 (s, 1H, 8-*H*). Anal. ($C_{26}H_{24}N_4O_3S$) C, H, N, S.

2-(3-Isopropoxyphenyl)-3-phenylamino-2H-pyrido[4,3-e]-1,2,-4-thiadiazine 1,1-dioxide (**27**). A suspension of 3-(1*H*-imidazol-1-yl)-2-(3-isopropoxyphenyl)-2*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide, **17** (0·3 g, 0·78 mmol) in aniline (2·0 mL) was heated under reflux for 1 h. Water (10 mL) was added, the pH was adjusted to 4 with formic acid, and the mixture was extracted with chloroform (50 mL). The organic layer was dried over $MgSO_4$, concentrated to small volume (5 mL) under reduced pressure and petroleum ether (30 mL) was added. The solid product so obtained was dissolved in a minimum volume of MeOH and treated with charcoal. Addition of three volumes of water induced the complete precipitation of the compound as white crystals (yield 31%). mp 138–143°C; IR (KBr) 3438, 3246 (N-H), 1627, 1597, 1569 (C=C, C=N, N-H), 1328, 1151 (S=O) cm^{-1}; ^1H NMR δ 1·1 (d, 6H, O-CH(C*H*$_3$)$_2$), 4·4 (m, 1H, O-C*H*(CH_3)$_2$), 6·35-7·4 (m, 9H, 2-*phenyl* + 3-NH-*phenyl*), 6·7 (d, 1H, 5-*H*), 7·85 (d, 1H, 6-*H*), 8·5 (s, 1H, 8-*H*), 9·6 (bs, 3-N-*H*). Anal. ($C_{21}H_{20}N_4O_3S$) C, H, N, S.

3-[(1-Methoxyphen-4-ylmethyl)amino]-2-phenyl-2H-pyrido[4,3-e]-1,2,4-thiadiazine 1,1-dioxide (**28**). This compound was obtained from 3-(1*H*-imidazol-1-yl)-2-phenyl-2*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide, **16**, and *p*-methoxybenzylamine by the procedure used to obtain **21** (yield 78%). mp 136–138°C; IR (KBr) 3425 (N-H), 1612, 1585, 1538, 1512 (C=C, C=N, N-H), 1336, 1166 (S=O) cm^{-1}; ^1H NMR δ 3·65 (s, 3H, O-C*H*$_3$), 4·5 (bs, 2H, 3-NH-C*H*$_2$-phenyl), 4·7 (bs, 1H, 3-N*H*-CH_2-phenyl), 6·95 (d, 2H, 3′-*H* + 5′-*H* methoxyphenyl), 7·0 (d, 2H, 2′-*H* + 6′-*H* methoxyphenyl), 7·1 (d, 1H, 5-*H*), 7·4 (s, 5H, 2-*phenyl*), 8·55 (d, 1H, 6-*H*), 8·8 (s, 1H, 8-*H*). Anal. ($C_{20}H_{18}N_4O_3S$) C, H, N, S.

2-(3-Isopropoxyphenyl)-3-[(1-methoxyphen-4-ylmethyl)amino]-2H-pyrido[4,3-e]-1,2,4-thiadiazine 1,1-dioxide (**29**). This compound was obtained from 3-(1*H*-imidazol-1-yl)-2-(3-isopropoxyphenyl)-2*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide, **17**, and 4-methoxybenzylamine by the procedure used to obtain 23. The compound was recrystallized from 1:2 $CHCl_3$-hexane and the precipitate was chromatographed (1:1 ethyl acetate-petroleum ether (bp 40–60°C), SiO_2) to give pure 29 (yield 70%). mp 163–166°C; IR (KBr) 3375 (N-H), 1610, 1585, 1554, 1512 (C=C, C=N, N-H), 1336, 1170 (S=O) cm^{-1}; ^1H NMR δ 1·25 (d, 6H, O-CH(C*H*$_3$)$_2$), 3·7 (s, 3H, O-C*H*$_3$), 4·5 (bm, 3H, O-C*H*(CH_3)$_2$ + 3-NHC*H*$_2$), 4·8 (bs, 1H, 3-N*H*), 6·6–7·4 (m, 9H, 5-*H* + 2-*phenyl*; + 3-NHCH_2-*phenyl*), 8·55 (d, 1H, 6-*H*), 8·8 (s, 1H, 8-*H*). Anal. ($C_{23}H_{24}N_4O_4S$) C, H, N, S.

3-[(1-Methoxyphen-2-ylmethyl)amino]-2-phenyl-2-H-pyrido[4,3-e]-1,2,4-thiadiazine 1,1-dioxide (**30**). This compound was obtained from 3-(1*H*-imidazol-1-yl)-2-phenyl-2*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide, 16, and *o*-methoxybenzylamine by the procedure used to obtain 21 (yield 80%). mp 159–161°C; IR (KBr) 3433 (N-H), 1609, 1600, 1544 (C=C, C=N, N-H), 1330, 1168 (S=O) cm^{-1}; ^1H NMR δ 3·5 (s, 3H, O-C*H*$_3$), 4·55 (d, 2H, 3-NHC*H*$_2$), 5·3 (bs, 1H, 3-N*H*), 6·7 (d, 1H, 3′-*H* methoxyphenyl), 6·85 (d, 1H, 5-*H*), 7·0–7·55 (m, 5H, 2-*phenyl* + 3-*methoxyphenyl*),8·5 (d, 1H, 6-*H*), 8·8 (s, 1H, 8-*H*). Anal. ($C_{20}H_{18}N_4O_3S$)C, H, N, S.

2-(3-Isopropoxyphenyl)-3-[(1-methoxyphen-2-ylmethyl)amino]-2H-pyrido[4,3-e]-1,2,4-thiadiazine 1,1-dioxide (**31**). This compound was obtained from 3-(1*H*-imidazol-1-yl)-2-(3-isopropoxyphenyl)-2*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide, **17**, and *o*-methoxybenzylamine by the procedure used to obtain **23**. Recrystallization from 1:2 $CHCl_3$-hexane furnished pure **31** (yield 76%). mp 146–149°C; IR (KBr) 3406 (N-H), 1605, 1587, 1538 (C=C, C=N, N-H), 1338, 1170 (S=O) cm^{-1}; ^1H NMR δ 1·2 (d, 6H, O-CH(C*H*$_3$)$_2$), 3·5 (s, 3H, O-C*H*$_3$), 4·35 (m, 1H, O-C*H*(CH_3)$_2$), 4·6 (d, 2H, 3-NHC*H*$_2$), 5·4 (bs, 1H, 3-N*H*), 6·4–7·4 (m, 9H, 5-*H* + 2-*phenyl* + 3-NHCH_2-*phenyl*), 8·55 (d, 1H, 6-*H*), 8·8 (s, 1H, 8-*H*). Anal. ($C_{23}H_{24}N_4O_4S$) C, H, N, S.

2-(3-Isopropoxyphenyl)-3-[2-(1-methoxyphen-4-ylethyl)amino]-2H-pyrido[4,3-e]-1,2,4-thiadiazine 1,1-dioxide (**32**). A solution of 3-(1*H*-imidazol-1-yl)-2-(3-isopropoxyphenyl)-2*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide, **17** (0·8 g, 2·1 mmol) and 4-methoxyphenethylamine (1·6 mL) in dioxane (8 mL) was heated under reflux for 1 h. The solvent was removed by distillation under reduced pressure and the residue was dispersed in water (20 mL). The solution was adjusted to pH 4 with formic acid and extracted with chloroform. After drying over $MgSO_4$, the organic phase was concentrated under reduced pressure and the oily residue was chromatographed (12:8 ethyl acetate-petroleum ether (bp 40–60°C), SiO_2). Recrystallization from 1:3 MeOH-H_2O furnished the pure compound (yield 70%). mp 50–65°C; IR (KBr) 3423 (N-H), 1607, 1553, 1534, 1512 (C=C, C=N, N-H), 1332, 1170 (S=O) cm^{-1}; ^1H NMR δ 1·3 (d, 6H, O-CH(C*H*$_3$)$_2$), 2·65 (t, 2H, 3-NHCH$_2$C*H*$_2$-phenyl), 3·55 (t, 2H, 3-NHC*H*CH$_2$-phenyl), 3·7 (s, 3H, O-C*H*$_3$), 4·5 (m, 2H, O-C*H*(CH_3)$_2$ + 3-N*H*), 6·4–7·4 (m, 9H, 5-*H* + 2-*phenyl* + 3-NHCH_2-*phenyl*), 8·55 (d, 1H, 6-*H*), 8·8 (s, 1H, 8-*H*). Anal. ($C_{24}H_{26}N_4O_4S.\frac{1}{2}H_2O$) C, H, N, S.

N-*[1,1-Dioxo-2-(3-isopropoxyphenyl)-2H-pyrido[4,3-e]-1,2,4-thiadiazin-3-yl]-l-tryptophan methyl ester* (**33**). L-tryptophan methyl ester hydrochloride (1·2 g, 4·7 mmol) was dissolved in water (10 mL) and adjusted to pH 12 with a 10% aqueous solution of NaOH. The resulting suspension was extracted twice with chloroform. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude L-tryptophan methyl ester residue and 3-(1*H*-imidazol-1-yl)-2-(3-isopropoxyphenyl)-2*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide, **17** (0·8 g, 2·1 mmol) were dissolved in dioxane (20 mL) and the mixture was heated under reflux for 15 h. The solvent was removed by distillation under reduced pressure and the residue dissolved in water (20 mL). The solution was adjusted to pH 4 with formic acid and the precipitate so obtained was collected by filtration, washed with water and

dried. The solid product was dissolved in the minimum volume of $CHCl_3$, treated with charcoal and three volumes of petroleum ether were added. The resulting precipitate was finally chromatographed (14:8 ethyl acetate–petroleum ether, SiO_2) to give the pure compound (yield 30%). mp 93–96°C; IR (KBr) 3397 (N-H), 1743 (C=O), 1604, 1549 (C=C, C=N, N-H), 1343, 1174 (S=O) cm^{-1}; ^1H NMR δ 1·2 (d, 6H, O-CH(CH_3)$_2$), 3·2 (bd, 2H, 3-NHCHCH_2-indole), 3·6 (s, 3H, O-CH_3), 4·25 (m, 1H, O-CH(CH_3)$_2$), 5·0 (bt, 1H, 3-NH$CH$$CH_2$-indole), 6·55–7·5 (m, 10H, 5-*H* + 2-*phenyl* + 3-NHCHCH_2-*indole*), 7·95 (bs, 1H, 3-N*H*), 8·55 (d, 1H, 6-*H*), 8·8 (s, 1H, 8-*H*), 10·6 (bs, 1H, N*H* indole). Anal. ($C_{27}H_{27}N_5O_5S$) C, H, N, S.

N-*(1,1-Dioxo-2-phenyl-2H-pyrido[4,3-e]-1,2,4-thiadiazin-3-yl)-l-tryptophan methyl ester (***34***).* This compound was obtained from 3-(1*H*-imidazol-1-yl)-2-phenyl-2*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide, 16, by the procedure used to obtain **33** (41%). mp 76–95°C; IR (KBr) 3397 (N-H), 1741 (C=O), 1607, 1549 (C=C, C=N, N-H), 1343, 1173 (S=O) cm^{-1}; ^1H NMR δ 3·2 (bd, 2H, 3-NHCHCH_2-indole), 3·6 (s, 3H, O-CH_3), 4·8 (bt, 1H, 3-NHCHCH_2-indole), 6·6–7·6 (m, 10H, 5-*H* + 2-phenyl + *3-NHCHCH*$_2$-indole), 7·95 (bs, 1H, 3-N*H*), 8·55 (d, 1H, 6-*H*), 8·8 (s, 1H, 8-*H*), 10·8 (bs, 1H, N*H* indole). Anal. ($C_{24}H_{21}N_5O_4S.H_2O$) C, H, N, S.

Cholecystokinin receptor-binding assays

The receptor-binding assays were conducted as described in the literature: CCK-B receptor binding was performed with male Swiss mouse cerebral cortex membranes according to the method of VanDijk et al (1984); CCK-A receptor binding affinity was determined in male Wistar rat pancreas following the method of Chang & Lotti (1986). For the CCK-A receptor the ligand was 20 pM [^{125}I]CCK-8 and incubation was at 37°C for 30 min. For the CCK-B receptor the ligand was 0.4 nm [^{3}H]CCK-8 and incubation was at 25°C for 20 min. For both receptors the non-specific ligand was CCK-8 (1 μM).

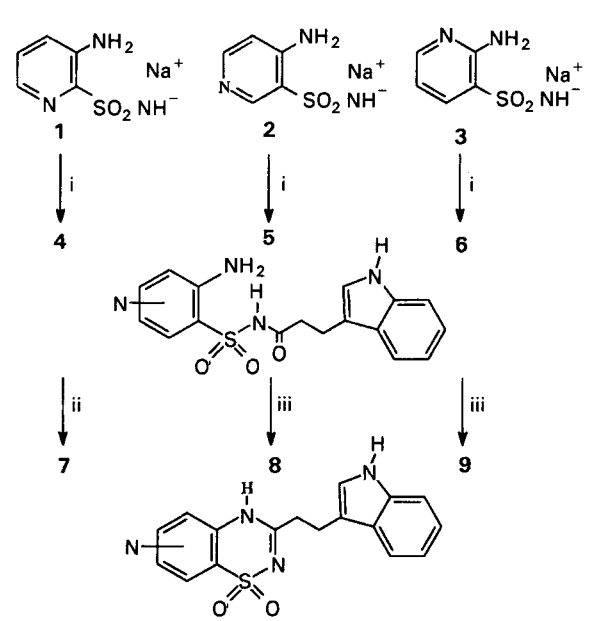
After the appropriate incubation period, the membranes were rapidly filtered (GF/B filters, Whatman) and washed several times by use of a 'Cell Harvester' (Brandel) filtration system. The filters were placed in vials containing the scintillation liquid (Formula 989, DuPont NEN) and the fixed radioactivity was measured with a Beckman LS6000 recorder.

The affinity of the compounds for the cholecystokinin receptors were evaluated by competition experiments. The molecules were tested on each receptor at concentrations of 1×10^{-5} M and 1×10^{-7} M (n=3). A validation competitive curve using CCK-8 at eight different concentrations (n=2) was performed for each experiment.

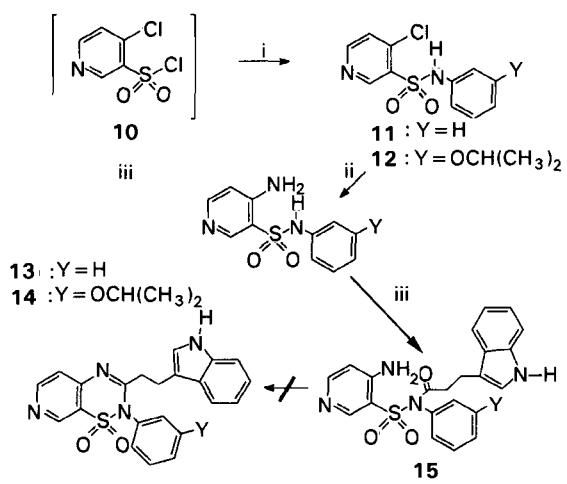
Chemistry

3-[2-(1*H*-Indol-3-ylethyl)]-4*H*-1,2,4-pyridothiadiazine 1,1-dioxides **7–9** bearing the pyridinic nitrogen atom in the 5-, 7- and 8-positions were prepared by reaction of succinimidyl (1*H*-indol-3-yl)propionate on the different aminopyridinesulphonamide sodium salts **1** (Lejeune et al 1984), **2** (Pirotte et al 1993) and **3** (West German Patent 1972). The resulting *N*-(aminopyridinesulphonyl)-3-(1*H*-indol-3-yl)propanamides **5** and **6** were cyclized by fusion to give the expected compounds (Scheme 2). Because fusion of the propanamide intermediate **4** gave very low yield, compound **7** was obtained by heating **4** in pyridine in the presence of a catalytic amount of pyridinium *p*-toluenesulphonate. 3-Aralkylamino-2-aryl-2*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides were obtained by following the synthetic pathway described in Scheme 3. The starting materials *N*-aryl-4-aminopyridine-3-sulphonamides **13** and **14** were prepared by the action of aniline or 3-isopropoxyaniline (Yu et al 1991) on 4-chloropyridine-3-sulphonyl chloride, **10**. Subsequent action of concentrated ammonia on **11** and **12** gave the corresponding aminopyridinesulphonamides. This type of compound easily reacted with the *N*-hydroxysuccinimide ester and led to the propanamide derivatives **15**. Unfortunately, all our attempts to prepare the expected product after ring closure were unsuccessful (Scheme 3). Moreover, except triethyl orthoformate, other carboxylic reagents failed to cyclize the *N*-substituted aminopyridinesulphonamides. As a result, we were not able to gain access to the strict analogues of quinazolinone cholecystokinin-ligands. We thus changed our synthetic pathway toward 3-aralkylamino compounds. The classical means of obtaining 3-(aryl)alkylaminopyrido- or benzothiadiazine dioxides (i.e. by treatment of the 3-methylsulphanyl intermediate with the appropriate amine, were unsuccessful (Raffa et al 1962; Hayao et al 1968; Bell et al 1975; Petersen 1973; Wollweber et al 1981; de Tullio et al 1996; cf. Scheme 4). *N*-substituted 4-aminopyridine-3-sulphonamides did not, however, easily cyclize with conventional reagents such as urea, phosgene, thiourea or thiophosgene to give the 3-oxo- or 3-thioxo-2-substituted pyridothiadiazine dioxides. 3-Oxo derivatives were obtained from 1,1′-carbonyldiimidazole alone (European Patent 1988).

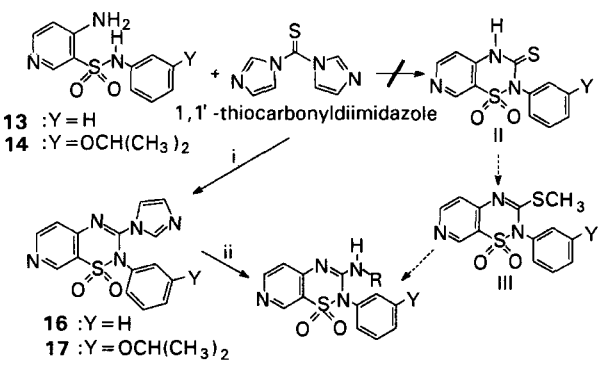
Unfortunately, thionation of the 3-oxo function of the 3-oxo derivatives to give the corresponding 3-thioxo derivatives did not occur. We then decided to use 1,1′-thiocarbonyldiimidazole as a ring-closure reagent for *N*-aryl-4-aminopyridine-3-sulphonamides (Scheme 4). 1,1′-Thiocarbonyldiimidazole was



SCHEME 2. Synthesis of 3-[2-(1*H*-indol-3-ylethyl)]-4*H*-pyrido[3,2-*e*] (**7**), [4,3-*e*] (**8**) and [2,3-*e*] (**9**) -1,2,4-thiadiazine 1,1-dioxides. Reagents: i, succinimidyl 3-(1*H*-indol-3-yl)propionate; ii, pyridinium *p*-toluenesulphonate, pyridine, reflux; iii, fusion.



SCHEME 3. Synthesis of *N*-aryl-4-aminopyridine-3-sulphonamides. Reagents: i, dioxane, triethylamine, aniline if Y = H and 3-isopropoxyaniline if Y = $OCH(CH_3)_2$; ii, NH_3c; iii, succinimidyl 3-(1*H*-indol-3-yl)propionate.

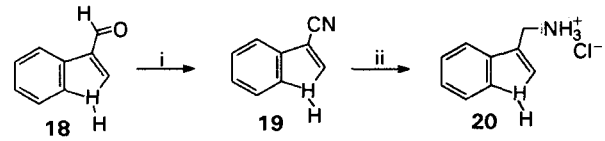


SCHEME 4. Synthesis of 3-aralkylamino-2-aryl-2*H*-pyrido[4,3-e]-1,2,4-thiadiazine 1,1-dioxides. Reagents: i, dioxane-DMF, reflux; ii, dioxane, aralkylamine, reflux.

recently employed as a thiophosgene analogue in the synthesis of 3-thioxo-1,2,4-benzothiadiazine 1,1-dioxides (Weller et al 1992). Surprisingly, its reaction with our intermediates **13** and **14** did not lead to the expected 3-thioxo derivatives II (Scheme 4) but to 3-(imidazol-1-yl)-substituted compounds **16** and **17**. These molecules appeared to be excellent substitutes for the 3-methylsulphanyl compounds III because they also reacted with different aralkylamines to give the expected 3-aralkylamino-2-aryl-2*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides **21** to **34** by loss of the imidazole leaving-group. 1*H*-Indol-3-ylmethylamine **20** which was used in the synthesis of compound **23** was obtained as the hydrochloride according to Scheme 5. (1*H*-Indol-3-yl)carbonitrile **19** was first prepared by reaction of diammonium hydrogen phosphate on indol-3-carboxaldehyde **18** (Organic Synthesis, coll. Vol. V, 656–657). Reduction of the nitrile function with lithium aluminium hydride led to the aminomethyl intermediate **20**.

Results and discussion

The different compounds synthesized were evaluated as CCK-A and CCK-B receptor ligands. The ability of the compounds



SCHEME 5. Synthesis of the 3-aminomethyl-1*H*-indole hydrochloride 20. Reagents: i, $(NH_4)_2HPO_4$, CH_3NO_2; ii, $LiAlH_4$, HCl.

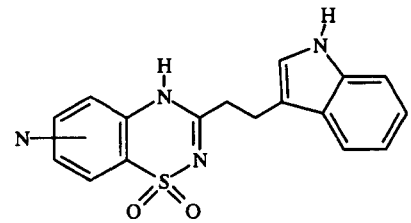
to displace radiolabelled CCK-8 from their specific binding sites was measured at concentrations of 10^{-5} and 10^{-7} M. CCK-8 is a natural octapeptide corresponding to the active part of cholecystokinin. Results were expressed as the percentage of CCK-8 specifically displaced from the cholecystokinin receptors at a determined drug concentration. None of the compounds was active at a concentration of 10^{-7} M.

As noted in Table 1, the 2-unsubstituted derivatives were inactive on both cholecystokinin receptor types, irrespective of the position of the pyridinic nitrogen atom. Thus, no structural information was provided about the most favourable nitrogen-atom position.

Table 2 reports the CCK-A receptor binding results of the 3-aralkylamino compounds **17** to **34**. The previously described quinazolinones were found to have very low affinity for CCK-A receptors even at a concentration of 10^{-5} M. Comparatively, our compounds at the same concentration had higher affinity for these cholecystokinin receptors, although their potencies were modest compared with those of the best CCK-A receptor antagonists such as devazepide, which exert activity in the sub-nanomolar range of concentration.

The CCK-B receptor binding results are presented in Table 2. As described in the literature, this receptor appeared to be the preferential biological target of the quinazolinone model (Yu et al 1991, 1992). According to the bioisostery between the quinazolinone and the pyridothiadiazine dioxide rings, we expected our compounds to have high affinity and selectivity for the CCK-B receptors. Unfortunately, none of the pyridothiadiazine dioxide isosteres were able to approach the potency of quinazolinones. Our best compounds had activity in the micromolar range of concentration, far from the sub-micromolar to the nanomolar concentration affinities of

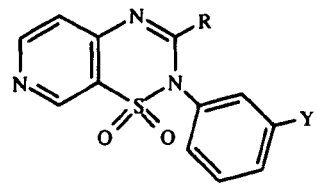
Table 1. CCK-A and CCK-B receptor binding data for 3-[2-(1*H*-indol-3-ylethyl)]-4*H*-1,2,4-pyridothiadiazine 1,1-dioxides.* (10^{-5})M



Compound	Position of pyridinic nitrogen atom	Receptor CCK-A†	CCK-B‡
7	8	–	–
8	7	12	16
9	5	–	10

*Standard errors were less than 15%. † Percent inhibition (10 μM) of ^{125}I-labelled CCK-8 sulphate binding with rat-pancreas membranes. ‡Percent inhibition (10 μM) of ^{3}H-labelled CCK-8 sulphate binding with mouse-brain membranes. Less than ten percent inhibition.

Table 2. CCK-A and CCK-B receptor binding data for 2-aryl-3-substituted-2*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides.* (10^{-5}M)



Compound	Y	R	Receptor CCK-A†	CCK-B‡
17	-$OCH(CH_3)_2$	1-imidazole	– §	–
21	H	$NHCH_2$-(phenyl)	–	31
22	-$OCH(CH_3)_2$	$NHCH_2$-(phenyl)	32	47
23	-$OCH(CH_3)_2$	$NHCH_2$-(indol-3-yl)	–	–
24	-$OCH(CH_3)_2$	$NHCH_2CH_2$-(indol-3-yl)	41	32
25	H	$NHCH_2$-(naphth-1-yl)	25	24
26	-$OCH(CH_3)_2$	$NHCH_2$-(naphth-1-yl)	17	–
27	-$OCH(CH_3)_2$	NH-phenyl	57	–
28	H	$NHCH_2$-(*p*-OCH_3-phenyl)	–	16
29	-$OCH(CH_3)_2$	$NHCH_2$-(*p*-OCH_3-phenyl)	17	35
30	H	$NHCH_2$-(*o*-OCH_3-phenyl)	–	25
31	-$OCH(CH_3)_2$	$NHCH_2$-(*o*-OCH_3-phenyl)	24	37
32	-$OCH(CH_3)_2$	$NHCH_2$CH2-(*p*-OCH_3-phenyl)	33	52
33	-$OCH(CH_3)_2$	tryptophan methyl ester	18	–
34	H	tryptophan methyl ester	41	36

*Standard errors were less than 15%. † Percent inhibition (10 μM) of ^{125}I-labelled CCK-8 sulphate binding with rat-pancreas membranes. ‡Percent inhibition (10 μM) of ^{3}H-labelled CCK-8 sulphate binding with mouse-brain membranes. §Less than ten percent inhibition.

recently described quinazolinone CCK-B antagonists. Such derivatives seemed to be equipotent or more potent than asperlicin and the old generation of cholecystokinin antagonists such as proglumide (Hahne et al 1981), lorglumide or benzotript (Makovec et al 1986).

Structure–activity relationships reported for quinazolinones clearly showed that minor modifications of the structure of the molecule and, more specifically, of the nature of the spacers connecting the two heteroaromatic domains could dramatically reduce the binding affinity to cholecystokinin receptors (Yu et al 1992). Thus, we could expect that slight modification of the structures of our 2- and 3-substituted pyridothiadiazine dioxides would lead to an increase in potency and affinity for cholecystokinin-receptors. We can, moreover, propose some hypotheses to explain the observed differences between the quinazolinone family of cholecystokinin antagonists and our compounds: substitution of the carbonyl group of quinazolinones by a sulphonyl group led to a strong increase in the negative potential centred on the oxygen atoms; replacement of the benzene nucleus (quinazolinone) with pyridine (pyridothiadiazine dioxide) also introduced an additional negative potential zone in the inferior part of the molecules; and the presence of a nitrogen atom on the linker connecting the two heteroaromatic domains could influence the geometry of the drugs.

In the near future, the synthesis and the biological evaluation of benzothiadiazine dioxides IV, of pyridopyrimidinones V and of 2-aralkylaminoquinazolinones VI (Fig. 2) are expected to identify the structural element responsible for the low activity of our compounds.

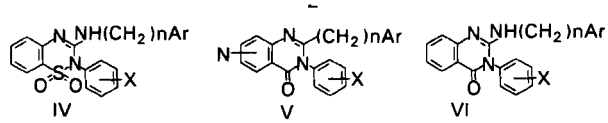


FIG. 2. New prospects in the design of CCK receptor ligands.

In conclusion, we have synthesized and characterized novel 3-aralkyl-4*H*-1,2,4-pyridothiadiazine 1,1-dioxides and a variety of 3-aralkylamino-2-aryl-2*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides. These molecules were tested as CCK-A and CCK-B receptor ligands and some appeared to be effective in the micromolar range of concentration. They were, however, less active than their quinazolinone isosteres. The discovery of the structural elements responsible for the loss of affinity will be the focus of future research. In addition to its pharmacological goal, this research has contributed to increasing our knowledge of the unusual chemistry of the 2,3-disubstituted-1,2,4-pyridothiadiazine 1,1-dioxides.

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