ELSEVIER

www.elsevier.nl/locate/farmac

Il Farmaco 55 (2000) 369–375

IL FARMACO

Synthesis of new anthranilic acid dimer derivatives and their evaluation on CCK receptors

Antonio Varnavas *, Lucia Lassiani, Valentina Valenta

Department of Pharmaceutical Sciences, University of Trieste, P.le Europa 1, 34127 Trieste, Italy

Received 20 February 2000; accepted 7 April 2000

Abstract

We have described previously an innovative bond disconnection strategy of asperlicin, a naturally occurring CCK receptor antagonist, leading to anthranilic acid dimer and tryptophan synthons. We have also demonstrated that when the tryptophan residue is connected to the C- or N-terminal sides of the anthranilic acid dimer, compounds with similar micromolar CCK-A receptor affinities are obtained. In order to investigate the binding effects of different N-terminal substitution, in this paper we describe a new series of anthranilic acid dimer derivatives, characterized by the presence of the tryptophan residue in the C-terminus of the dimer. Among the compounds synthesized, the *N*-1*H*-indol-3-propionyl derivative exhibited an improved, at the micromolar range, affinity for the CCK-A receptor in comparison to that of either, the N-unsubstituted derivative and asperlicin. The lead compound emerging from this key step of our investigation represents the new starting point for the development of a new class of CCK-A receptor ligands. © 2000 Elsevier Science S.A. All rights reserved.

Keywords: CCK receptor ligands; Anthranilic acid dimer

---

1. Introduction

Cholecystokinin (CCK) is a polypeptide hormone found in the gastrointestinal tract as well as in the brain [1]. CCK is distributed in various biologically active molecular forms throughout the peripheral tissues and central nervous system (CNS), being the C-terminal octapeptide (CCK-8) the minimal sequence required for bioactivity [2,3].

The major hormonal effects of CCK are expressed in the gastrointestinal tract where CCK stimulates the gall bladder contraction, the pancreatic exocrine secretion and increases the intestinal motility [4–6].

At the CNS, CCK acts as a neurotransmitter/neuromodulator and appears to be involved in anxiety, panic, depression, nociception and satiety [7–11].

The biological effects of CCK are mediated by at least two distinct G-protein-coupled receptors. Both receptors are present in the periphery and in the CNS, although CCK-A receptors occur predominantly at the periphery (gastrointestinal tract) and CCK-B receptors are located mainly in the CNS [12].

In view of the importance of CCK in different physiopathological processes as irritable bowel syndrome (IBS), chronic and acute pancreatitis, gastric ulcer, anxiety, panic disorder, eating disorders etc., over the past 10 years, great efforts have been made to develop clinically useful receptor CCK-ligands [13].

At least two chemical classes (1,4-benzodiazepines, quinazolinones) of potent non-peptide and selective antagonists for the CCK-A and CCK-B receptors have been discovered starting from the natural product asperlicin (Fig. 1) after a sequence of chemical semplifications and/or modifications [14–17].

Recently, we have proposed anthranilic acid dimer as a useful template for the development of a new class of CCK-receptor ligands. The above flexible non-peptide template derives from asperlicin as a result of an innovative bond disconnection strategy which preserves the tryptophan residue and the aromatic domains but the other heteroaromatic bicyclic substructures [18]. Moreover, as in the case of the peptoid derivatives, anthranilic acid dimer offers the possibility to be derivatized either at the N-terminal and/or C-terminal site [19,20].

---

* Corresponding author. Tel.: +39-040-676 7861; fax: +39-040-525 72.

E-mail address: varnavas@univ.trieste.it (A. Varnavas).

PII: S0014-827X(00)00053-7

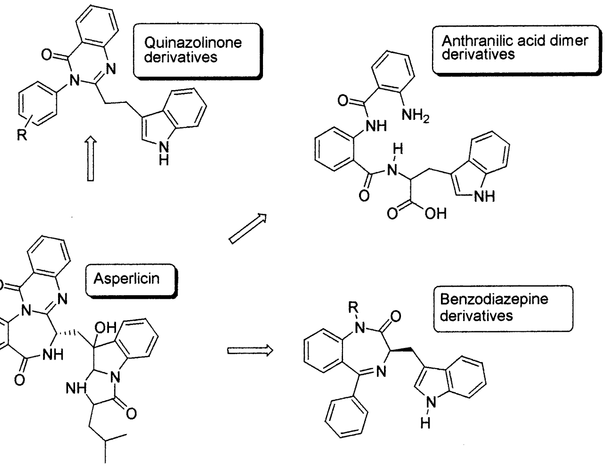
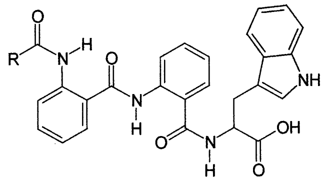


Fig. 1. Structures of lead compounds derived from different disconnection approaches of the natural CCK receptors antagonist asperlicin.

Table 1
Structure of the target compounds

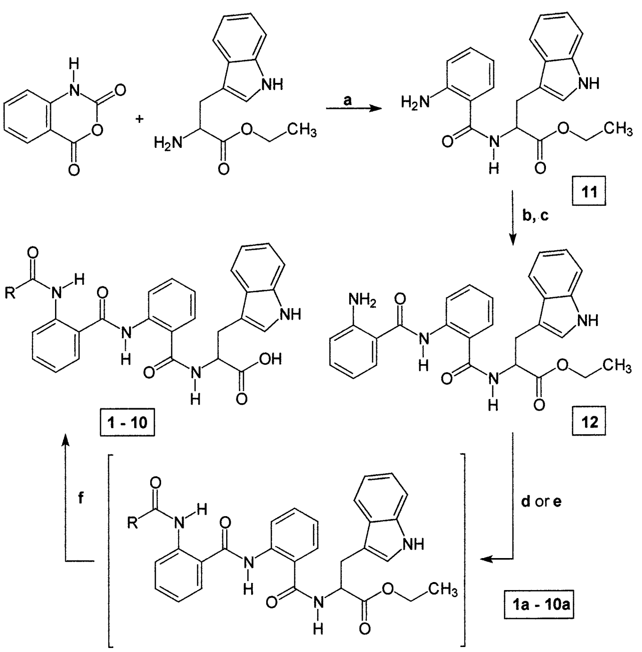


Comp.	R
1	1*H*-indol-3-yl-$(CH_2)_2$–
2	1*H*-indol-3-yl-CH_2–
3	1*H*-indol-2-yl-
4	C_6H_5–
5	C_6H_4-*p*-Cl
6	C_6H_5–CH_2–
7	C_6H_5–$(CH_2)_2$–
8	C_6H_5–CH=CH–
9	HOOC–CH_2–
10	HOOC–$(CH_2)_2$–

We have also demonstrated that the introduction of the tryptophan residue, which represents one of the synthons of the disconnection scheme, either at the N- or C-terminal site of anthranilic acid dimer, led to compounds with similar micromolar CCK-A receptor affinities ($IC_{50} \approx 7$ μM) [21].

Taking into account the behavior of these anthranoyl-anthranilic acid derivatives and trying to optimize their CCK-A affinity, we have prepared a series of N-substituted anthranilic acid dimer derivatives holding the tryptophan residue at the C-terminal site of dimer. At the present optimization step, the substituents selected to be appended at the N-terminus of the biologically inert molecular scaffold are the indol-3-yl or indol-2-yl moieties as well as a phenyl ring linked to the dimer, via amide bond formation, either directly or by a short alkyl chain (compounds **1**–**8**). The choice of these pharmacophoric groups is strongly conditioned by the hypothesis that the presence of a second lipophilic pocket on the receptor could involve these moieties in hydrophobic, charge transfer or, in the case of the indole, hydrogen-bonding interactions. Moreover, the insertion of a free carboxylic group (compounds **9**, **10**) was directed to investigate the binding in the presence of an additional ionic function at the opposite part of the molecule (N-terminus).

The new compounds, reported in Table 1, were evaluated as CCK receptor ligands by determining their binding affinity for CCK-A and CCK-B receptors.



Scheme 1. (a) AcOEt, Et_3N; (b) 2-nitrobenzoyl chloride, CH_2Cl_2, Et_3N; (c) Zn, CH_3COOH, CH_2Cl_2; (d) RCOOH, *iso*butyl chloroformate, CH_2Cl_2, Et_3N; (e) RCOCl, CH_2Cl_2, Et_3N; (f) KOH, MeOH.

2. Chemistry

Scheme 1 summarizes the synthetic pathway used to obtain the compounds listed in Table 1. The reaction of the tryptophan ethyl ester with isatoic anhydride afforded the intermediate **11** in 80% yield. Acylation of anthranoyl derivative **11** with *o*-nitrobenzoyl chloride and subsequent reduction of the nitro group provided the anthranilic acid dimer derivative **12**. Carboxylic acids were activated by the mixed anhydrides method or via the acyl chloride formation and subsequently coupled with the key intermediate **12** to afford compounds **1a**–**3a**, whereas compounds **4a**–**10a** were obtained via commercially available acyl chlorides.

Finally, the alkaline hydrolysis of esters **1a**–**10a** afforded almost quantitatively the target compounds **1**–**10**, of which the synthetic data are listed in Tables 2–4.

3. Biochemistry

Compounds **1**–**10** (Table 1) were tested for their ability to displace [^{3}H]-(±)-L-364,718 and [^{3}H]-(+)-L-365,260 from their specific binding in rat pancreatic and guinea pig brain membranes, respectively [22,23].

The IC_{50} values were calculated from five-point inhibition curves by log–probit plots and the reported values are the geometric means of at least three separate experiments. Compounds exhibiting at 10 μM, a percentage of inhibition (I%) lower than 20% were considered inactive.

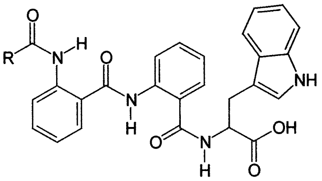
4. Results and discussion

The affinities of the synthesized compounds for the CCK-A and CCK-B receptors were evaluated by receptor binding assays and are shown in Table 5. In general, none of the investigated compounds showed appreciable percentage of inhibition towards CCK-B receptors at a concentration of 10 μM. By contrast, most of the tested compounds exhibited binding affinities for the CCK-A receptors in the micromolar range. Among these, compounds **1**–**3** having an indole ring at the N-terminus of the anthranilic acid dimer showed similar or more potent affinity for CCK-A receptors compared with that of the naturally occurring CCK antagonist asperlicin ($IC_{50} = 1.4 \times 10^{-6}$ M) [24].

Moreover, the most potent one (compound **1**) showed some sevenfold greater affinity than that of the N-unsubstituted anthranilic acid dimer derivative compound **0**. Compound **2**, in which the alkyl chain (ethyl group) of the substituent of the parent compound **1** was shortened to a methyl group, exhibited threefold less potent activity than **1**. The introduction of the indol-2-yl moiety at the N-terminus of compound **0** provided compound **3** which was comparable to compound **1** with regard to their affinity. Phenyl substituted compounds (compounds **4**–**8**) reversed this effect and displayed a decreased potency and selectivity for CCK-A receptor compared with those bearing the indole ring. Furthermore, no significant differences in CCK-A receptor affinity were observed between the parent compound **4** and compounds **6** and **7** where the alkyl chain of the substituent was lengthened. The introduction of an electron-withdrawing group, such as chloro, at the 4-position of the phenyl ring of the substituent (compound **5**) as well as the unsaturation of the alkyl linker (compound **8**) had little effect on receptor affinity. On the other hand, the introduction of an acidic side chain (compounds **9** and **10**) is detrimental for biological activity.

These data suggest that for the indole ring containing substituents an additional binding interaction appears to be present. The increased affinity observed for these compounds suggest also that appropriate steric interac-

Table 2
Physicochemical properties of compounds **1**–**10**



Comp.	R	Molecular formula	M_W	Crystal solvent [a]	R_f	M.p. (°C)
1	3-Indolyl-$(CH_2)_2$–	$C_{36}H_{31}N_5O_5$	611	A	0.40 [b]	190–191
2	3-Indolyl-CH_2–	$C_{35}H_{29}N_5O_5$	599	A	0.35 [b]	194–196
3	2-Indolyl	$C_{34}H_{27}N_5O_5$	585	A	0.24 [b]	228–230
4	Phenyl	$C_{32}H_{26}N_4O_5$	546	B	0.30 [b]	175–177
5	4-Chlorophenyl	$C_{32}H_{25}ClN_4O_5$	580.5	C	0.23 [b]	232–234
6	Benzyl	$C_{33}H_{28}N_4O_5$	560	A	0.24 [b]	114–117
7	Phenylethyl	$C_{34}H_{30}N_4O_5$	574	C	0.32 [b]	169–170
8	Phenylethenyl	$C_{34}H_{28}N_4O_5$	572	C	0.34 [b]	165–168
9	HOOC–CH_2–	$C_{28}H_{24}N_4O_7$	528	A	0.27 [c]	152–155
10	HOOC–$(CH_2)_2$–	$C_{29}H_{26}N_4O_7$	542	A	0.24 [d]	193–195

[a] Crystallization solvents: A, MeOH; B, EtOH; C, MeOH–Et_2O.
[b] AcOEt–MeOH: 3/1.
[c] AcOEt–MeOH: 1/1.
[d] AcOEt–MeOH: 3/2.

Table 3
^{1}H NMR (DMSO-d_6) of compounds **1**–**10** [a]

Comp.	δ (ppm)
1	2.73 (t, 2H, –CH$_2$–CO–); 3.00 (t, 2H, –C*H*$_2$–CH$_2$–CO–); 3.27 (m, 2H, >CH–C*H*$_2$–); 4.68 (m, 1H, >CH–); 6.85–8.52 (m, 18H, ar); 9.08 (d, 1H, –N*H*–CH<); 10.72 (s, 1H, –NH– ind); 10.77 (s, 1H, –NH– ind); 10.83 (s, 1H, –NH–); 11.96 (s, 1H, –NH–).
2	3.10 (m, 2H, –C*H*$_2$–CH<); 3.74 (s, 2H, –CH$_2$–CO–); 4.65 (m, 1H, –CH<); 6.85–8.50 (m, 18H, ar); 9.15 (d, 1H, –N*H*–CH<); 10.65 (s, 1H, –NH– ind); 10.70 (s, 1H, –NH– ind); 11.50 (s, 1H, –NH–); 12.10 (s, 1H, –NH–).
3	3.17 (m, 2H, –C*H*$_2$–CH<); 4.65 (m, 1H, >CH–); 6.93–8.57 (m, 18H, ar); 8.93 (d, 1H, –N*H*–CH<); 10.80 (s, 1H, –NH– ind); 11.92 (s, 1H, –NH– ind); 11.97 (s, 1H, –NH–); 12.30 (s, 1H, –NH–).
4	3.08 (m, 2H, –CH$_2$–); 4.58 (m, 1H, >CH–); 6.90–8.55 (m, 18H, ar); 8.85 (d, 1H, –N*H*–CH<); 10.75 (s, 1H, –NH– ind); 11.85 (s, 1H, –NH–); 12.15 (s, 1H, –NH–).
5	3.24 (m, 2H, –CH$_2$–); 4.70 (m, 1H, >CH–); 6.95–8.60 (m, 17H, ar); 9.08 (d, 1H, –N*H*–CH<); 10.82 (s, 1H, –NH– ind); 11.75 (s, 1H, –NH–); 12.18 (s, 1H, –NH–).
6	3.25 (m, 2H, –C*H*$_2$–CH<); 3.68 (s, 2H, –CH$_2$–CO–); 4.52 (m, 1H, >CH–); 6.84–8.47 (m, 18H, ar); 8.65 (d, 1H, –N*H*–CH<); 10.71 (s, 1H, –NH– ind); 10.83 (s, 1H, –NH–); 12.15 (s, 1H, –NH–).
7	2.63 (t, 2H, –CH$_2$–CO–); 2.86 (t, 2H, C$_6$H$_5$–C*H*$_2$–); 3.17 (m, 2H, –C*H*$_2$–CH<); 4.68 (m, 1H, >CH–); 6.96–8.60 (m, 18H, ar); 9.05 (d, 1H, –N*H*–CH<); 10.60 (s, 1H, –NH– ind); 10.82 (s, 1H, –NH–); 11.95 (s, 1H, –NH–).
8	3.30 (m, 2H, –CH$_2$–); 4.65 (m, 1H, >CH–); 6.85–8.52 (m, 20H, ar and –CH=CH–); 8.95 (d, 1H, –N*H*–CH<); 10.80 (s, 1H, –NH– ind); 10.91 (s, 1H, –NH–); 12.10 (s, 1H, –NH–).
9	3.28 (m, 2H, –C*H*$_2$–CH<); 3.46 (s, 2H, –CH$_2$–CO); 4.74 (m, 1H, >CH–); 6.95–8.57 (m, 13H, ar); 9.10 (d, 1H, –N*H*–CH<); 10.85 (s, 1H, –NH– ind); 10.98 (s, 1H, –NH–); 12.03 (s, 1H, –NH–).
10	2.54 (t, 2H, –NHCO–C*H*$_2$–); 2.70 (t, 2H, –C*H*$_2$–COOH); 3.32 (m, 2H, –C*H*$_2$–CH<); 4.68 (m, 1H, >CH–); 6.95–8.56 (m, 13H, ar); 9.06 (d, 1H, –N*H*–CH<); 10.71 (s, 1H, –NH– ind); 10.82 (s, 1H, –NH–); 12.00 (s, 1H, –NH–).

[a] Abbreviations: ar, aromatic; ind, indole; s, singlet; d, doublet; t, triplet; m, multiplet.

tions, lipophilicity and hydrogen bond formation of the N-terminus substituent may be important for fitting this part of the molecule into a lipophilic pocket on the receptor.

5. Conclusions

We have reported a new series of N-substituted anthranilic acid dimer derivatives starting from the lead compound **0** which derived from a new disconnection strategy of asperlicin. An increased affinity for CCK-A receptors was achieved by introducing an indole moiety at the N-terminus of the starting compound. The lead compounds (compounds **1** and **3**) established from this key step of our investigation represents the new starting point for the development of a new class of CCK-A receptor ligands. Further investigations are needed to optimize the binding interactions of the N-terminus substituents of this lead compound in order to confer a strong affinity for CCK-A receptors. Moreover, it is reasonable to expect that further improvements in activity can be achieved by optimizing the distance and the spatial arrangements of the N- and C-terminal pharmacophores of the anthranilic acid dimer. For this reason additional research to explore this and other possibilities is in progress.

6. Experimental

6.1. Chemistry

Melting points were determined on a Büchi 510 melting point apparatus and are uncorrected. Proton (^{1}H NMR, 200 MHz) and carbon (^{13}C NMR, 50 MHz) nuclear magnetic resonance spectra were recorded on a Varian-Gemini 2000 Fourier transform spectrometer. Chemical shifts are reported in δ (ppm) relative to tetramethylsilane (TMS) as internal standard. The following abbreviations are used throughout: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; ar, aromatic and ind, indole. Spectral data are consistent with assigned structures. All reactions were routinely checked by ascending thin-layer chromatography (TLC) on precoated silica gel plates (60F-254 Merck).

6.1.1. N-Anthranoyl-DL-tryptophan ethyl ester (**11**)

DL-Tryptophan ethyl ester hydrochloride (5.37 g; 20 mmol) was suspended in ethyl acetate (250 ml) and treated with triethylamine (2.81 ml; 20 mmol) followed by isatoic anhydride (3.26 g; 20 mmol). The suspension was refluxed for 2 h, cooled to room temperature (r.t.) and filtered. The organic phase was shaken in a separator flask with 1 M NaOH (2 × 50 ml), water (2 × 50 ml), dried over anhydrous sodium sulfate and filtered. The filtrate was evaporated to dryness under reduced pressure and the resulting oily residue was triturated with petroleum ether (40–60°; 300 ml). The analytically pure title compound which precipitated was collected by filtration, washed with petroleum ether (40–60°) and dried (5.7 g; 81%).

R_f 0.49 (AcOEt–hexane: 1/1); m.p. 98–100°C; ^{1}H NMR (CDCl$_3$): δ 1.24 (t, 3H, –CH$_3$); 3.46 (d, 2H, >CH–C*H*$_2$–); 4.16 (q, 2H, –CH$_2$–O–); 5.06 (m, 1H, >CH–); 5.49 (s, 2H, –NH$_2$); 6.52–7.60 (m, 10H, ar, –NH–); 8.11 (s, 1H, –NH– ind). ^{13}C NMR (CDCl$_3$): δ 14.14, 27.71, 53.26, 61.61, 110.08, 111.32, 115.49, 116.67, 117.27, 118.69, 119.51, 119.66, 122.17, 122.24, 122.92, 127.60, 132.53, 148.79, 168.96.

Table 4
^{13}C NMR (DMSO-d_6) of compounds **1**–**10**

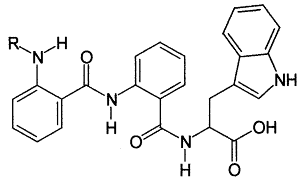
Comp.	δ (ppm)
1	20.46, 26.29, 37.66, 53.57, 110.00, 111.10, 111.24, 113.10, 117.90, 117.97, 118.03, 118.17, 120.43, 120.69, 121.65, 122.03, 122.94, 123.34, 123.45, 126.73, 126.86, 127.32, 128.24, 131.97, 132.09, 135.89, 135.99, 138.07, 138.53, 166.05, 168.36, 170.71, 172.82.
2	34.20, 36.09, 53.95, 109.12, 111.43, 112.05, 113.95, 116.88, 117.87, 118.05, 118.23, 120.35, 120.55, 121.47, 122.10, 123.00, 123.30, 123.80, 126.40, 126.73, 127.25, 128.15, 131.70, 132.23, 135.67, 136.00, 137.95, 138.35, 165.80, 168.15, 169.85, 172.65.
3	26.44, 54.05, 102.76, 110.31, 111.17, 112.30, 117.97, 118.07, 119.95, 120.65, 120.92, 121.07, 121.43, 121.70, 123.24, 123.34, 123.86, 126.82, 127.06, 127.38, 128.19, 131.33, 132.11, 132.63, 135.89, 136.93, 138.34, 139.05, 159.12, 166.65, 168.13, 172.97.
4	26.57, 54.47, 110.61, 111.09, 117.94, 118.03, 120.54, 121.02, 121.36, 122.10, 123.00, 123.25, 123.60, 126.60, 126.86, 127.23, 127.42, 128.02, 128.70, 131.82, 131.94, 132.50, 134.35, 135.83, 138.33, 139.02, 164.54, 166.57, 167.61, 173.19.
5	26.33, 53.58, 110.02, 111.23, 117.88, 118.16, 120.66, 120.74, 120.84, 121.70, 122.78, 123.16, 123.40, 123.88, 126.91, 127.45, 128.30, 128.75, 128.84, 132.13, 132.42, 133.15, 135.93, 136.65, 138.38, 138.56, 163.62, 166.44, 168.31, 172.75.
6	26.66, 43.90, 55.21, 110.95, 111.15, 117.74, 118.16, 120.35, 120.50, 121.16, 121.53, 122.92, 123.09, 123.28, 123.43, 126.46, 127.17, 127.55, 127.75, 128.15, 129.16, 131.54, 131.84, 134.96, 135.77, 138.10, 138.46, 165.92, 167.37, 169.06, 173.95.
7	26.33, 30.46, 38.12, 53.56, 110.03, 111.23, 117.89, 118.17, 120.58, 120.74, 121.74, 122.96, 123.39, 123.54, 125.71, 126.91, 127.31, 127.98, 128.04, 128.23, 131.91, 132.04, 135.93, 137.88, 138.51, 141.00, 166.04, 168.33, 170.17, 172.75.
8	26.41, 53.87, 110.23, 111.18, 117.95, 118.09, 120.68, 120.87, 120.96, 121.82, 122.17, 123.10, 123.33, 123.58, 127.04, 127.27, 127.86, 128.17, 128.69, 129.70, 131.93, 132.01, 134.33, 135.90, 138.27, 138.40, 140.79, 163.48, 166.13, 168.16, 172.90.
9	26.31, 53.56, 110.04, 111.21, 117.90, 118.15, 120.62, 120.72, 121.77, 123.02, 123.37, 123.70, 126.92, 127.24, 128.22, 131.91, 132.01, 135.91, 137.59, 138.45, 164.55, 165.83, 168.29, 168.79, 172.76.
10	26.30, 28.57, 31.48, 53.56, 110.03, 111.22, 117.90, 118.16, 120.52, 120.74, 121.47, 122.95, 123.27, 123.39, 126.88, 127.31, 128.24, 132.08, 135.88, 138.10, 138.48, 166.07, 168.32, 169.92, 172.80, 173.39.

*6.1.2. N-(N-Anthranoyl)anthranoyl-*DL*-tryptophan ethyl ester (**12**)*

A solution of *N*-anthranoyl-DL-tryptophan ethyl ester (**11**) (17.52 g; 50 mmol) and triethylamine (7.03 ml; 50 mmol) in dry dichloromethane (250 ml) was cooled at 0°C and stirred during the dropwise addition of a solution of 2-nitrobenzoyl chloride (9.25 g; 50 mmol) in dry dichloromethane (60 ml). After stirring for 2 h at r.t. the reaction mixture was washed in succession with 1 M NaOH (2 × 30 ml) and water (2 × 30 ml). The organic layer was dried over anhydrous sodium sulfate and filtered. The filtrate was evaporated under reduced pressure to give the crude nitro derivative, which was used immediately without further purification in the next step of the synthesis. The nitro derivative was dissolved in dichloromethane (100 ml) and treated with 10 g of zinc dust. The resulting suspension was cooled at 0°C and, within 10 min, 12 ml of glacial acetic acid was added dropwise with stirring. The mixture was stirred for 1 h at r.t. and then filtered. The organic phase was washed with 1 M NaOH (2 × 50 ml) and water (2 × 50 ml), dried over anhydrous sodium sulfate and evaporated. Crystallization from 90% ethanol afforded the analytically pure compound **12** (16.2 g; 69%).

R_f 0.45 (AcOEt–hexane: 1/1); m.p. 150°C; ^1H NMR ($CDCl_3$): δ 1.25 (t, 3H, $-CH_3$); 3.43 (m, 2H, $>CH-CH_2-$); 4.17 (q, 2H, $-O-CH_2-$); 5.08 (m, 1H, $-CH<$); 5.76 (s, 2H, $-NH_2$); 6.68–7.69 (m, 12H, ar); 8.30 (s, 1H, –NH– ind); 8.65 (d, 1H, ar); 11.69 (s, 1H, –NH–). ^{13}C NMR ($CDCl_3$): δ 14.19, 27.65, 53.53, 61.86, 109.76, 111.42, 115.97, 117.10, 117.51, 118.53, 119.75, 120.51, 121.67, 122.33, 122.77, 122.92, 126.97, 127.61, 127.86, 132.67, 132.85, 136.14, 139.80, 149.68, 168.00, 168.70, 171.68.

Table 5
CCK receptors binding data



Comp.	R	CCK-A (%) [a] 1×10^{-5} M	CCK-B (%) [b] 1×10^{-5} M
0	H	(7.0×10^{-6} M) [c]	IN [d]
1	1*H*-indol-3-yl-$(CH_2)_2$–CO–	(1.1×10^{-6} M) [c]	IN [d]
2	1*H*-indol-3-yl-CH_2–CO–	(3.2×10^{-6} M) [c]	IN [d]
3	1*H*-indol-2-yl–CO–	(1.8×10^{-6} M) [c]	IN [d]
4	C_6H_5–CO–	60	40
5	C_6H_4–*p*-Cl–CO–	75	35
6	C_6H_5–CH_2–CO–	65	IN [d]
7	C_6H_5–$(CH_2)_2$–CO–	58	45
8	C_6H_5–CH=CH–CO–	53	38
9	HOOC–CH_2–CO–	44	IN [d]
10	HOOC–$(CH_2)_2$–CO–	IN [d]	IN [d]

[a] Percentage of inhibition at 10 μM of [^3H]-(±)-L-364,718 binding in rat pancreatic membranes.

[b] Percentage of inhibition at 10 μM of [^3H]-(+)-L-365,260 in guinea pig brain membranes.

[c] IC_{50} (μM) given as the mean of at least three independent determinations. The maximum standard error was always less than 20% of the geometric mean.

[d] Inactive, percentage of inhibition less than 20% at 10 μM.

6.1.3. General procedure for the synthesis of N-[N-(substituted)anthranoyl] anthranoyl-DL-tryptophan ethyl ester derivatives (**1a–2a**)

A solution of 10 mmol of the corresponding acid in dry dichloromethane (100 ml) cooled at −10°C was treated with triethylamine (1.4 ml; 10 mmol) followed by *iso*butyl chloroformate (1.31 ml; 10 mmol). The resulting mixture was stirred at −10°C for 20 min and treated dropwise with a solution of compound **12** (4.7 g; 10 mmol) in 50 ml of dry dichloromethane. After the addition was complete, the reaction was stirred at r.t. for 1 h and then refluxed for 3 h. The solvents were evaporated, the residue was dissolved in dichloromethane and the organic phase was washed with diluted aqueous sodium hydroxide solution and water. The organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was used without further purification in the next step of the synthesis.

6.1.4. General procedure for the synthesis of N-[N-(substituted)anthranoyl] anthranoyl-DL-tryptophan ethyl ester derivatives (**3a–10a**)

A solution of 10 mmol of the corresponding acyl chloride (the indole-2-carbonyl chloride was prepared according to the procedure described by Kermack [25]) in 30 ml of dry dichloromethane was added gradually to a solution of compound **12** (4.7 g, 10 mmol) in the same solvent (50 ml). The pH of the reaction was adjusted to 9.5 with triethylamine and the solution stirred for 2 h. The reaction mixture was diluted with 100 ml of dichlomethane and washed in succession with 0.1 N sodium hydroxide solution, water, 0.1 N hydrochloric acid and water. The dried (sodium sulfate) organic phase was evaporated under reduced pressure to give a crude residue, which was used without further purification in the next step of the synthesis.

6.1.5. General procedure for the preparation of compounds **1–10**

A mixture of 5 mmol of the corresponding ester (compounds **1a**–**10a**) and potassium hydroxide (0.56 g; 10 mmol) in methanol (50 ml) was warmed gently for 4 h. The solvent was removed under reduced pressure and the residue taken up with water. After cooling, the solution was adjusted to pH 2–3 with diluted HCl. The resulting milky white suspension was extracted with AcOEt (2 × 50 ml), and the combined organic extracts were dried over sodium sulfate and evaporated to give the corresponding title compound. Crystallization from the appropriate solvent afforded the analytically pure derivatives **1**–**10** (Table 2).

Spectroscopic ^{1}H NMR and ^{13}C NMR data are presented in Tables 3 and 4, respectively.

6.2. Biological evaluation

6.2.1. General

Male rats (Wistar) and male guinea pigs (Hartley) were obtained from Charles River, Calco, Como (Italy). [^{3}H]-(±)-L-364,718 and [^{3}H]-(+)-L-365,260 were purchased from NEN Research products (Brussels) with specific activities of 87 and 75.3 Ci/mmol, respectively. (*R*,*S*)-L-364,718 and (*R*,*S*)-L-365,260 were synthesized in our laboratory as described previously [26]. Radioactivity was counted with 4 ml of Aquassure (NEN) high performance LSC cocktail in a Packard TRI-CARB 300 liquid scintillator.

6.2.2. Binding studies

All experiments were performed in triplicate.

CCK-A receptor binding of [^{3}H]-(±)-L-364,718 was performed as described previously [22]. Briefly, samples of 0.4 ml containing 0.8 mg of wet tissue (≈7.2 μg/ml protein) were incubated for 30 min at 37°C in the presence of [^{3}H]-L-364,718 (0.2 nM final concentration) and a solution of various concentrations of the compounds to be tested. The buffer used for binding assay was 50 mM Tris–HCl (pH 7.4 at 37°C), 5 mM $MgCl_2$, 5 mM dithiothreitol, 2 mg/ml of bovine serum albumin and 0.14 mg/ml bacitracin. The samples were then filtered under reduced pressure using glass fiber GF/B (Whatman) filters and rinsed four times with 4 ml of cooled Tris buffer (50 mM, pH 7.4). Non-specific binding was determined in the presence of (*R*,*S*)-L-364,718 (0.3 μM final concentration) and was always less than 10% of the total binding.

CCK-B receptor affinities were determined by displacement of [^{3}H]-(+)-L-365,260 from guinea pig cerebral cortex membranes as described previously by Chang et al. [23]. The buffer used was 10 mM HEPES, 5 mM $MgCl_2$, 1 mM EGTA, 130 mM NaCl and 0.25 mg/ml bacitracin, pH 6.5 and the glass fiber filters were GF/C (Whatman). Briefly, displacement experiments were performed by incubation of 0.5 ml of brain membranes corresponding to 6.2 mg of wet tissue (200 μg/ml protein) for 30 min at 25°C in the presence of [^{3}H]-(+)-L-365,260 (1 nM final concentration) plus various concentrations of the compounds to be tested. Non-specific binding was determined in the presence of (*R*,*S*)-L-365,260 (2 μM final concentration). Specific binding was defined as the radioactivity after subtracting non-specific binding and was 45% of total.

References

[1] J.A. Williams, Cholecystokinin: a hormone and a neurotransmitter, Biomed. Res. 3 (1982) 107–115.

[2] J.R. Reeve, V. Eysselein, J.H. Walsh, C.M. Ben Avram, J.E. Shively, New molecular forms of cholecystokinin. Microse-

quence analysis of forms previously characterized by chromatographic methods, J. Biol. Chem. 261 (1986) 16392–16397.

[3] L.I. Larsson, J.F. Rehfeld, Localization and molecular heterogeneity of cholecystokinin in the central and peripheral nervous systems, Brain Res. 165 (1979) 201–218.

[4] R.T. Jensen, S.A. Wank, W.H. Rowley, S. Sato, J.D. Gardner, Interaction of CCK with pancreatic acinar cells, Trends Pharmacol. Sci. 10 (1989) 418–423.

[5] R.A. Liddle, B.J. Gertz, S. Kanayama, L. Beccaria, L.D. Coker, T.A. Turnbull, E.T. Morita, Effects of a novel cholecystokinin (CCK) receptor antagonist, MK-329, on gallbladder contraction and gastric emptying in humans. Implications for the physiology of CCK, J. Clin. Invest. 84 (1989) 1220–1225.

[6] B.M. Meyer, B.A. Werth, C. Beglinger, P. Hildebrand, J.B. Jansen, D. Zach, L.C. Rovati, G.A. Stalder, Role of cholecystokinin in regulation of gastrointestinal motor functions, Lancet 2 (1989) 12–15.

[7] O. Brawman Mintzer, R.B. Lydiard, J. Bradwejn, G. Villarreal, R. Knapp, N. Emmanuel, M.R. Ware, Q. He, J.C. Ballenger, Effects of the cholecystokinin agonist pentagastrin in patients with generalized anxiety disorder, Am. J. Psychiatry 154 (1997) 700–702.

[8] H.J. van-Megen, J.A. den-Boer, H.G. Westenberg, On the significance of cholecystokinin receptors in panic disorder, Prog. Neuropsycho-pharmacol. Biol. Psychiatry 18 (1994) 1235–1246.

[9] C. Lofberg, H. Agren, J. Harro, L. Oreland, Cholecystokinin in CSF from depressed patients: possible relations to severity of depression and suicidal behaviour, Eur. Neuropsycopharmacol. 8 (1998) 153–157.

[10] F. Benedetti, M. Amanzio, C. Casadio, A. Oliaro, G. Maggi, Blockade of nocebo hyperalgesia by the cholecystokinin antagonist proglumide, Pain 71 (1997) 135–140.

[11] F.X. Pi-Sunyer, H.R. Kissileff, J. Thornton, G.P. Smith, C-terminal octapeptide of cholecystokinin decrease food intake, Physiol. Behav. 29 (1982) 627–630.

[12] C.T. Dourish, D.R. Hill, Classification and function of CCK receptors, Trends Pharmacol. Sci. 8 (1987) 207–209.

[13] P. de Tullio, J. Delarge, B. Pirotte, Recent advances in the chemistry of cholecystokinin receptor ligands (agonists and antagonists), Curr. Med. Chem. 6 (1999) 433–455.

[14] R.S. Chang, V.J. Lotti, R.L. Monaghan, J. Birnbaum, E.O. Stapley, M.A. Goetz, G. Albers-Schonberg, A.A. Patchett, J.M. Liesch, O.D. Hensens, J.P. Springer, A potent nonpeptide cholecystokinin antagonist selective for peripheral tissues isolated from *Aspergillus alliaceus*, Science 230 (1985) 177–179.

[15] B.E. Evans, M.G. Bock, K.E. Rittle, R.M. Di Pardo, W.L. Whitter, D.F. Veber, P.S. Anderson, R.M. Freidinger, Design of potent, orally effective, nonpeptidal antagonists of the peptide hormone cholecystokinin, Proc. Natl Acad. Sci. USA 83 (1986) 4918–4922.

[16] M.G. Bock, R.M. Di Pardo, B.E. Evans, K.E. Rittle, W.L. Whitter, D.F. Veber, P.S. Anderson, R.M. Freidinger, Benzodiazepine gastrin and brain cholecystokinin receptor ligands: L-365,260, J. Med. Chem. 32 (1989) 13–16.

[17] M.J. Yu, K.J. Thrasher, J.R. McCowan, N.R. Mason, L.G. Mendelsohn, Quinazolinone cholecystokinin-B receptor ligands, J. Med. Chem. 34 (1991) 1505–1508.

[18] A. Varnavas, L. Lassiani, E. Luxich, M. Zacchigna, Anthranoyl-anthranilic acid: a template for the development of a new class of cholecystokinin receptor ligands, Pharmazie 51 (1996) 697–700.

[19] J.M. Eden, M. Higginbottom, D.R. Hill, D.C. Horwell, J.C. Hunter, K. Martin, M.C. Pritchard, S.S. Rahman, R.S. Richardson, E. Roberts, Rationally designed dipeptoid analogues of cholecystokinin (CCK): N-terminal structure-affinity relationships of alpha-methyl tryptophan derivatives, Eur. J. Med. Chem. 28 (1993) 37–45.

[20] P.R. Boden, J.M. Eden, M. Higginbottom, D.R. Hill, D.C. Horwell, J.C. Hunter, K. Martin, M.C. Pritchard, R.S. Richardson, E. Roberts, Rationally designed dipeptoid analogues of cholecystokinin (CCK): C-terminal structure-activity relationships of alpha-methyl tryptophan derivatives, Eur. J. Med. Chem. 28 (1993) 47–61.

[21] A. Varnavas, L. Lassiani, E. Luxich, V. Valenta, C-terminal anthranoyl-anthranilic acid derivatives and their evaluation on CCK receptors, Farmaco 55 (2000) 293–302.

[22] R.S.L. Chang, V.J. Lotti, T.B. Chen, K.A. Kunkel, Characterization of the binding of [^{3}H]-(±)-L-364,718: a new potent, nonpeptide cholecystokinin antagonist radioligand selective for peripheral receptors, Mol. Pharmacol. 30 (1986) 212–217.

[23] R.S. Chang, T.B. Chen, M.G. Bock, R.M. Freidinger, R. Chen, A. Rosegay, V.J. Lotti, Characterization of the binding of [^{3}H]-L-365,260: a new potent and selective brain cholecystokinin (CCK-B) and gastrin receptor antagonist radioligand, Mol. Pharmacol. 35 (1989) 803–808.

[24] M.G. Bock, R.M. Di Pardo, K.E. Rittle, B.E. Evans, R.M. Freidinger, D.F. Veber, R.S.L. Chang, T.B. Chen, M.E. Keegan, V.J. Lotti, Cholecystokinin antagonists. Synthesis of asperlicin analogues with improved potency and water solubility, J. Med. Chem. 29 (1986) 1941–1945.

[25] W.O. Kermack, W.H. Perkin, R. Robinson, Harmin and harmaline. Part V. The synthesis of norharman, J. Chem. Soc. 119 (1921) 1602–1642.

[26] M.G. Bock, R.M. Di Pardo, B.E. Evans, K.E. Rittle, D.F. Veber, R.M. Freidinger, J. Hirshfield, J.P. Springer, Synthesis and resolution of 3-amino-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-ones, J. Org. Chem. 52 (1987) 3232–3239.