ELSEVIER

www.elsevier.nl/locate/farmac

Il Farmaco 55 (2000) 293–302

IL FARMACO

C-terminal anthranoyl–anthranilic acid derivatives and their evaluation on CCK receptors

Antonio Varnavas *, Lucia Lassiani, Elena Luxich, Valentina Valenta

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

Received 10 February 1999; accepted 1 September 1999

Abstract

A series of C-terminal anthranoyl–anthranilic acid derivatives arising from a strict bond disconnection approach of asperlicin were synthesized and examined for their CCK receptor affinities. These compounds represent the second step of our investigation directed toward the search for alternative substructures of asperlicin as a starting point for the development of a new class of CCK ligands. The obtained micromolar affinities for CCK-A rather than CCK-B receptor confirm that the anthranilic acid dimer represents a useful template for the development of selective CCK-A receptor ligands. © 2000 Elsevier Science S.A. All rights reserved.

Keywords: CCK ligands; Anthranilic acid dimer

1. Introduction

Cholecystokinin (CCK) is an endogenous peptide that occurs in both the gastrointestinal tract and central nervous system (CNS) [1]. Among its different molecular forms the C-terminal octapeptide (CCK-8) represents the minimum sequence required for bioactivity [2].

The entire range of the biological functions of CCK appears to be mediated by two receptors subtypes, CCK-A and CCK-B [3,4]. CCK-A receptors predominate in the periphery and mediate the hormone-like CCK activity (stimulation of gall bladder contraction and pancreatic enzyme secretion), but were also found in CNS [5,6].

CCK-B receptors are widely distributed in CNS and are thought to be involved in the control of anxiety and nociception [7–10].

The therapeutic potential of CCK ligands in treating gastrointestinal CCK-related disorders and in pathophysiological situations concerning different CCK-modulated central neural pathways, has stimulated an extensive research in this area and up to now manifold classes of potent and selective antagonists have been described [11–17].

Among these, non-peptide derivatives deserve a particular mention in consideration of their favourable in vivo stability. Many classes of these ligands could be related to substructures recognised in the molecule of asperlicin, the first non-peptide selective antagonist for CCK-A receptors isolated from *Aspergillus alliaceus* [13].

As part of our interest in non-peptide CCK receptor ligands, we have directed our efforts toward the search for alternative substructures of asperlicin as a starting point for the development of a new class of ligands.

We recently described an innovative bond disconnection of asperlicin, summarized in Fig. 1, showing that the structure of this natural product may be viewed as the result of intramolecular cyclization of the anthranilic acid dimer and tryptophan [18].

The derivatization of the N-terminal part of the anthranilic acid dimer, per se inactive, led to compounds with micromolar affinities for the CCK-A receptor subtype.

In this paper we wish to report the second and obvious step of our investigation concerning the synthesis and CCK receptor binding of C-substituted anthranoyl–anthranilic acid derivatives (Table 1).

* 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)00043-4

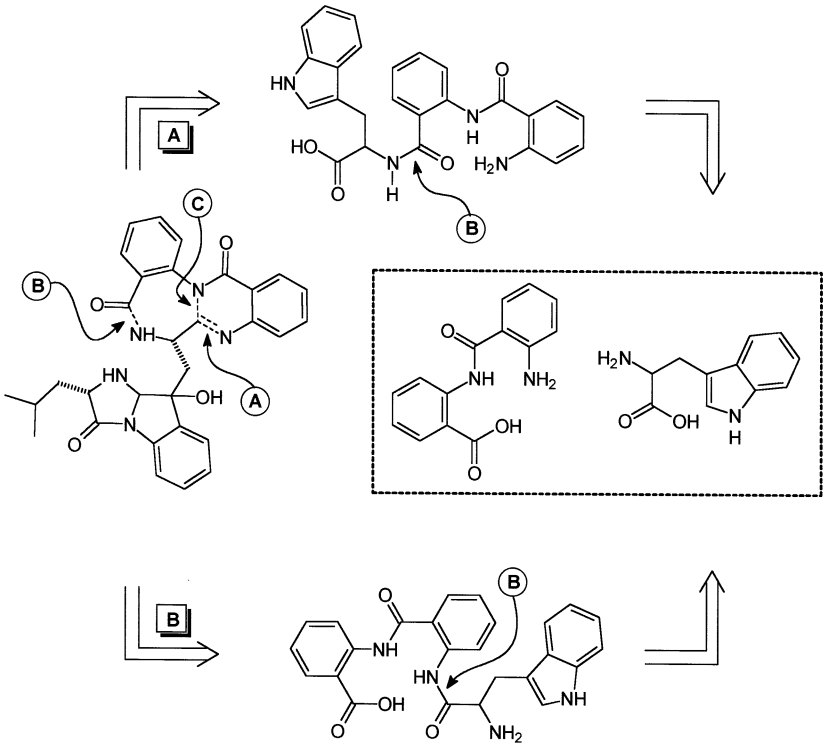


Fig. 1. Disconnection bond strategy. Path A: cut C followed by cut A both on amidinic group. Path B: cut C (amidinic group) followed by cut B (C–N amide). The preliminary bond disconnection of the fused leucine residue and FGI (dehydration) on the indolenine moiety is common to path A and B.

In consideration of the synthons obtained from the disconnection scheme, the substituents chosen are essentially amino acids of the C-terminus of CCK and already shown to be crucial for the interaction at the receptors.

2. Chemistry

The synthesis of the C-substituted antranoyl–anthranilic acid derivatives **1**–**25** was accomplished by standard procedures as depicted in Scheme 1.

Briefly, the *o*-aminobenzoyl derivatives (compounds **1a**–**10a** and **24a**–**25a**) were obtained in almost quantitative yield by reacting isatoic anhydride with the C-protected or with the decarboxylated (Trp and Phe) DL-amino acids, respectively (route a).

A further one-pot *o*-aminobenzoylation reaction performed as above on these intermediates (**1a**–**10a** and **24a**–**25a**) was excluded because of either steric hindrance and poor nucleophilicity toward isatoic anhydride.

Hence, we accomplished an *o*-nitrobenzoylation (route b) followed by reduction (route c) to obtain compounds **1**–**10** and **24**–**25**.

The subsequent alkaline hydrolysis of the esters **1**–**10** afforded almost quantitatively the target compounds **11**–**20** (route d) and similarly the ammonolysis of esters **1**, **2** and **8** gave the corresponding amides **21**–**23** (route e).

3. Biochemistry

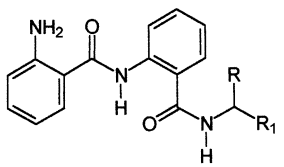
Compounds **1**–**25** (Table 1) were tested for their ability to displace [^{3}H]-(±)-L-364,718 and [^{3}H]-(+)-L-365,260 from their specific binding in rat pancreatic and guinea pig brain membranes, respectively [19,20].

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 a percentage of inhibition (I%) at 30 μM less than 20% were considered inactive.

4. Results and discussion

The reported C-substituted anthranoyl–anthranilic acid derivatives showed preferences for the CCK-A receptor resulting inactive against CCK-B. Furthermore, as previously observed for the N-substituted anthranoyl–anthranilic acid derivatives, only compounds with a free C-terminal carboxylic group exhibited receptor affinity [18]. In fact the corresponding esters (compounds **1**–**10**), as well as the amides (com-

Table 1
Structure of the target compounds



Comp.	R	R_1
1	$-CH_2$–3-indolyl	$-COOC_2H_5$
2	$-CH_2-C_6H_5$	$-COOC_2H_5$
3	–H	$-COOC_2H_5$
4	$-C_6H_5$	$-COOC_2H_5$
5	$-CH(CH_3)_2$	$-COOC_2H_5$
6	$-CH_2-CH(CH_3)_2$	$-COOC_2H_5$
7	$-(CH_2)_2-CH_3$	$-COOCH_3$
8	$-(CH_2)_3-CH_3$	$-COOCH_3$
9	$-(CH_2)_2-S-CH_3$	$-COOC_2H_5$
10	$-CH_2-COOC_2H_5$	$-COOC_2H_5$
11	$-CH_2$–3-indolyl	–COOH
12	$-CH_2-C_6H_5$	–COOH
13	–H	–COOH
14	$-C_6H_5$	–COOH
15	$-CH(CH_3)_2$	–COOH
16	$-CH_2-CH(CH_3)_2$	–COOH
17	$-(CH_2)_2-CH_3$	–COOH
18	$-(CH_2)_3-CH_3$	–COOH
19	$-(CH_2)_2-S-CH_3$	–COOH
20	$-CH_2-COOH$	–COOH
21	$-CH_2$–3-indolyl	$-CONH_2$
22	$-CH_2-C_6H_5$	$-CONH_2$
23	$-(CH_2)_3-CH_3$	$-CONH_2$
24	$-CH_2$–3-indolyl	–H
25	$-CH_2-C_6H_5$	–H

pounds **21**–**23**) resulted inactive. The CCK-A receptor binding data of compounds **11**–**20** are summarized in Table 11.

Compound **11**, a tryptophan derivative, showed the greater affinity inside this series. It is interesting to note that a similar binding behaviour was observed with the Trp residue connected at the N-terminus of the anthranilic acid dimer [18]. This fact well agrees with our disconnection strategy applied to asperlicin nucleus.

The observed binding data suggest a strong dependence of the affinity on the side chain of the amino acids employed. In particular nor-Val, nor-Leu, Met and Phe (compounds **17**–**19** and **12**) exhibited similar affinity and are characterized by a linear aliphatic side chain or by the presence of a benzyl group. On the other hand, the presence of branched aliphatic chains (compounds **15**, **16**), the shortening of the Phe residue (compound **14**) and particularly the absence of any residue (compound **13**) dramatically reduce the affinity.

As a result of this analysis it can be pointed out that the amino acid side chain represents a pharmacophoric group involved in receptor recognition.

Moreover and in support of our initial choice, inside this series, derivatives (**11**, **12**, **19**) having amino acids of the C-terminal tetrapeptide of CCK (i.e. Trp-Met-Asp-Phe-NH2) exhibited the highest degree of affinity with the only exception of compound **20**.

Another structural feature that strongly affects the receptor affinity concerns the presence of a carboxylic group since either derivatization (esters and amides) or decarboxylation (compounds **24** and **25**) led to inactive compounds. These facts could be ascribed to the lack of specific ionic interactions at the receptor (esters, amides and decarboxylated derivatives) and/or to unfavourable steric interactions between the pharmacophoric group and receptor site arising from a modified spatial arrangement as a result of decarboxylation.

5. Conclusions

In this paper we have reported the synthesis and biological evaluation of compounds representing the second step of anthranoyl–anthranilic acid derivatization according to the disconnection approach of asperlicin we proposed.

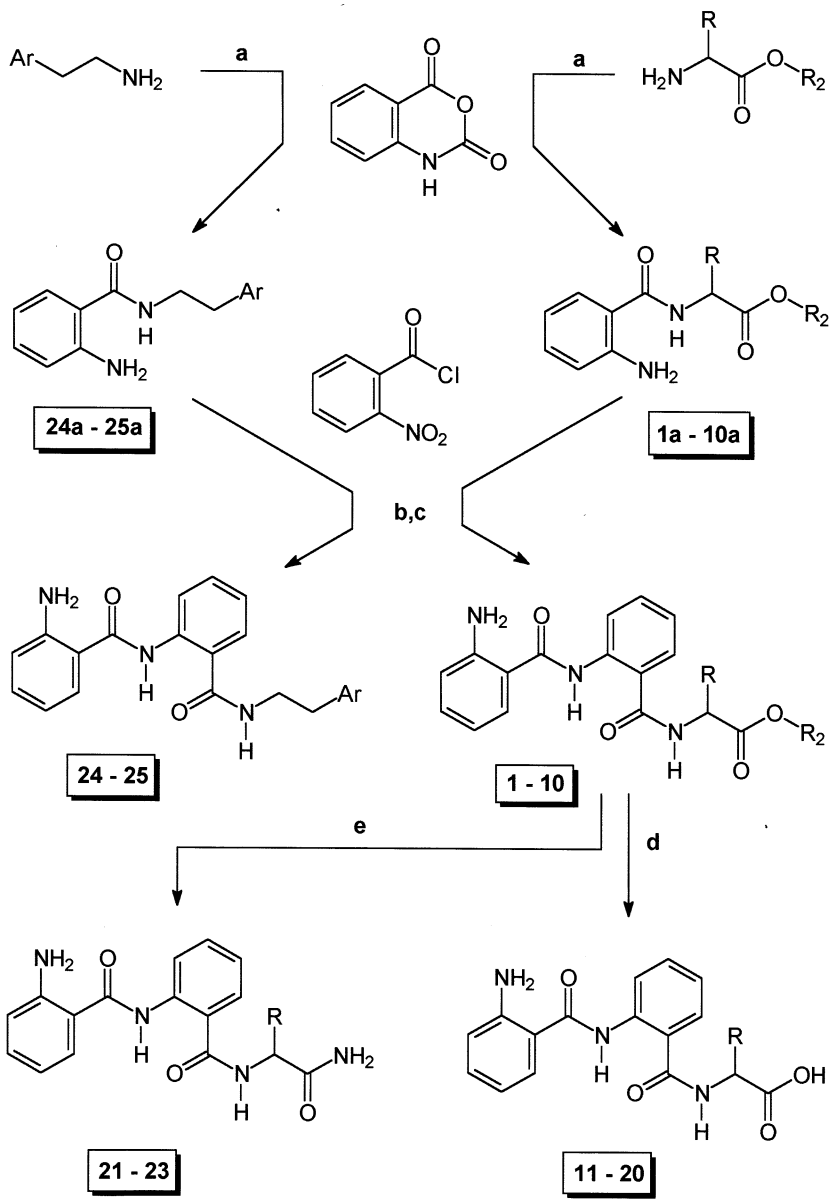
It appears that the N- as well as the C-substitution of anthranilic acid dimer, employed as template for stepwise optimization, with different amino acids, led to compounds that prefer CCK-A receptor with affinity of the same order of magnitude of asperlicin ($IC_{50} = 1.4$ μM [21]), the starting point of our investigation.

Further investigation demonstrating a higher affinity for the same receptor of compounds obtained by the simultaneous substitution of this template will be discussed in forthcoming papers and additional studies are in progress to explore the SAR of the central nucleus.

6. Experimental

6.1. Chemistry

Melting points were determined on a Büchi 510 melting point apparatus (Büchi, Flawil, Switzerland) and are uncorrected. Ascending thin-layer chromatography (TLC) was performed on precoated silica gel plates (60F-254 Merck) by using UV light to visualize the chromatograms. Proton (^{1}H NMR, 200 MHz) and carbon (^{13}C NMR, 50 MHz) nuclear magnetic resonance spectra were recorded on a Varian-Gemini 200 Fourier Transform spectrometer. Chemical shifts are reported as δ (ppm) relative to tetramethylsilane (TMS) as internal standard and the signals are described as s = singlet, d = doublet, dd = double doublet, t = triplet, q = quartet, b = broad and m = multiplet. Spectral data are consistent with assigned structures.



Scheme 1. (a) AcOEt, Et_3N; (b) CH_2Cl_2, Et_3N; (c) Zn, CH_3COOH, CH_2Cl_2; (d) KOH, MeOH; (e) NH_3(gas), MeOH.

6.1.1. General procedure for the preparation of the anthranilamides ***1a–10a*** *and* ***24a–25a***

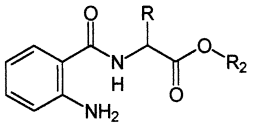
A suspension of 20 mmol of the corresponding two-substituted ethyl amine (phenylethylamine and tryptamine) or of the appropriate hydrochloride salt of the C-protected DL-amino acid in 500 ml of ethyl acetate was treated with triethylamine (2.81 ml, 20 mmol) followed by isatoic anhydride (3.26 g, 20 mmol). The resulting mixture was refluxed under stirring for 2 h, cooled down to room temperature (r.t.) and filtered. The organic phase was thoroughly washed with a 1 M NaOH aqueous solution (2 × 50 ml), water (2 × 50 ml), dried over anhydrous sodium sulphate and concentrated in vacuo. Trituration with petroleum ether (40–70°C) afforded the analytically pure title compounds.

Physicochemical properties and spectroscopic (^{1}H and ^{13}C NMR) data of compounds **1a–10a** are presented in Tables 2–4, respectively.

*6.1.1.1. N-anthranoyltryptamine (**24a**).* Compound **24a** was prepared according to the general procedure described earlier in 83% yield; m.p. 159–161°C; R_f 0.43 (1:1 AcOEt–hexane); ^{1}H NMR (DHSO): δ 2.96, t-2H (–CH_2–); 3.53, q-2H (–CH_2–NH–); 6.45, s-2H (–NH_2); 6.48–8.40, m-10H (arom., –NH–), 10.84, s-1H (–NH–, indole). ^{13}C NMR (DHSO): δ 25, 111, 112, 114, 115, 116, 118, 118.1, 121, 122, 127, 131, 136, 149, 169.

*6.1.1.2. N-anthranoyl-2-phenylethylamine (**25a**).* The compound was obtained in 82% yield as an oil. R_f 0.65

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



Comp.	R	R_2	Molecular formula	MW	Yield (%)	R_f [a]	M.p. (°C)
1a	–CH_2–3-indolyl	–C_2H_5	$C_{20}H_{21}N_3O_3$	351	81	0.49	98–100
2a	–CH_2–C_6H_5	–C_2H_5	$C_{18}H_{20}N_2O_3$	312	79	0.69	83–84
3a	–H	–C_2H_5	$C_{11}H_{14}N_2O_3$	222	66	0.52	70–72
4a	–C_6H_5	–C_2H_5	$C_{17}H_{18}N_2O_3$	298	42	0.72	81–83
5a	–$CH(CH_3)_2$	–C_2H_5	$C_{14}H_{20}N_2O_3$	264	65	0.70	48
6a	–CH_2–$CH(CH_3)_2$	–C_2H_5	$C_{15}H_{22}N_2O_3$	278	75	0.73	49–50
7a	–$(CH_2)_2$–CH_3	–CH_3	$C_{13}H_{18}N_2O_3$	250	65	0.61	87
8a	–$(CH_2)_3$–CH_3	–CH_3	$C_{14}H_{20}N_2O_3$	264	25	0.64	55
9a	–$(CH_2)_2$–S–CH_3	–C_2H_5	$C_{14}H_{20}N_2O_3S$	296	70	0.63	53–55
10a	–CH_2–$COOC_2H_5$	–C_2H_5	$C_{15}H_{20}N_2O_5$	308	59	0.56	89–90

[a] 1:1 AcOEt–hexane.

(1:1 AcOEt–hexane); ^{1}H NMR ($CDCl_3$): δ 2.91, t-2H (–CH_2–); 3.65, q-2H (–CH_2–NH–); 5.4, s-2H (–NH_2); 6.19, m-1H (–NH–); 6.55–7.37, m-9H (arom.). ^{13}C NMR ($CDCl_3$): δ 36, 41, 116.6, 117, 126.6, 127, 128.7, 129, 132, 139, 149, 169.

*6.1.2. General procedure for the preparation of the N-(N-anthranoyl) anthranilamides **1**–**10***

A solution of 20 mmol of the corresponding anthranilamides (**1a**–**10a**) in 100 ml of dry dichloromethane cooled at 0°C was treated with triethylamine (2.81 ml, 20 mmol) followed by 2-nitro-benzoyl chloride (3.70 g, 20 mmol). The resulting mixture was stirred at r.t. for 2 h. The reaction mixture was washed in succession with a 1 M NaOH (2 × 30 ml) and water (2 × 30 ml). The dried (sodium sulphate) organic phase was concentrated to give the crude nitro derivative, which was used without further purification. The residue was taken up with 100 ml of dichloromethane 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), water (2 × 50 ml), dried over sodium sulphate, and evaporated. Crystallization from the appropriate solvent afforded the analytically pure derivatives **1**–**10** (Table 5). Spectroscopic ^{1}H and ^{13}C NMR data are presented in Tables 6 and 7, respectively.

N-(*N*-anthranoyl)anthranoyltryptamine (**24**) and *N*-(*N*-anthranoyl)anthranoyl-2-phenylethylamine (**25**) were prepared according to the method described earlier. Crystallization from methanol afforded the pure title compounds in 54 and 48% yield, respectively.

Table 3
^{1}H NMR ($CDCl_3$) of compounds **1a**–**10a**

Comp.	δ (ppm)
1a	1.24, t-3H (–CH_3); 3.46, d-2H (–CH_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 (arom. –NH–); 8.11, s-1H (–NH–, indole).
2a	1.24, t-3H (–CH_3); 3.21, m-2H (–CH_2–CH<); 4.18, q-2H (–CH_2–O–); 4.97, q-1H (>CH–); 5.45, s-2H (–NH_2); 6.52, d-1H (–NH–); 6.61–7.28, m-9H (arom.).
3a	1.26, t-3H (–CH_3); 4.18, m-4H (–CH_2–O–, –CH_2–); 5.41, s-2H (–NH_2); 6.57–7.40, m-5H (arom. –NH–).
4a	1.23, t-3H (–CH_3); 4.20, q-2H (–CH_2–); 5.03, s-2H (–NH_2); 5.70, d-1H (–CH<); 6.64, m-2H (–NH–, H arom.); 7.10–7.43, m-8H (arom.).
5a	0.99, d-6H (–CH–(CH_3)$_2$); 1.30, t-3H (–CH_3); 2.25, m-1H (–CH–(CH_3)$_2$); 4.22, q-2H (–O–CH_2–); 4.70, m-1H (–NH–CH<); 5.12, s-2H (–NH_2); 6.57, d-1H (–NH–CH<); 6.67–7.44, m-4H (arom.).
6a	0.97, d-6H (>CH–CH_3); 1.27, t-3H (–CH_2–CH_3); 1.67, m-3H (>CH–CH_2–); 4.19, q-2H (–O–CH_2–); 4.76, m-3H (–CH–NH–, –NH_2); 6.52, d-1H (–NH–); 6.59–7.40. m-4H (arom.).
7a	0.94, t-3H (–CH_3); 1.42, m-2H (–CH_2–CH_3); 1.87, m-2H (>CH–CH_2–); 3.76, s-3H (–O–CH_3); 4.75, m-1H (–CH<); 5.29, s-2H (–NH_2); 6.57, d-1H (–NH–CH<); 6.68–7.41, m-4H (arom.).
8a	0.88, t-3H (–CH_3); 1.33, m-4H (–(CH_2)$_2$–CH_3); 1.85, m-2H (>CH–CH_2–); 3.76, s-3H (–O–CH_3); 4.74, m-1H (–CH<); 5.40, s-2H (–NH_2); 6.56, d-1H (–NH–CH<); 6.68–7.41, m-4H (arom.).
9a	1.30, t-3H (–CH_2–CH_3); 2.10, s-3H (–S–CH_3); 2.07, m-2H (>CH–CH_2); 2.57, t-2H (–CH_2–S–); 4.23, q-2H (–CH_2–CH_3), 4.83, m-3H (–CH<, –NH_2); 6.61–7.42, m-5H (arom. –NH–).
10a	1.24, t-3H (–CH_3); 1.28, t-3H (–CH_3); 3.00, m-2H (–CH_2–); 4.13, q-2H (–O–CH_2–); 4.24, q-2H (–O–CH_2–); 4.76, s-2H (–NH_2); 4.97, m-1H (–CH<); 6.62, d-1H (>CH–NH–); 6.68–7.42, m-4H (arom.).

Table 4
^{13}C NMR ($CDCl_3$) of compounds **1a**–**10a**

Comp.	δ (ppm)
1a	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.
2a	14.19, 38.05, 53.20, 61.63, 115.41, 116.69, 117.29, 127.15, 127.43, 128.60, 129.43, 132.59, 136.04, 148.84, 168.66, 171.75.
3a	14, 42, 62, 115, 116, 117, 128, 133, 149, 169, 170.
4a	14, 57, 62, 116.6, 117.3, 127.2, 127.6, 128.5, 129, 133, 137, 149, 168, 171.
5a	14.28, 17.99, 19.05, 31.61, 57.11, 61.37, 115.83, 116.72, 117.30, 127.42, 132.52, 148.73, 169.02, 172.22.
6a	14.20, 22.15, 22.88, 25.03, 41.85, 50.93, 61.42, 115.86, 116.65, 117.29, 127.44, 132.52, 148.77, 168.93, 173.34.
7a	13.75, 18.73, 34.72, 52.14, 52.41, 115.45, 116.67, 117.34, 127.45, 132.58, 148.81, 168.92, 173.40.
8a	13.89, 22.38, 27.49, 32.35, 52.28, 52.39, 115.43, 116.62, 117.31, 127.44, 132.56, 148.86, 168.86, 173.36.
9a	14, 16, 30, 32, 52, 62, 115, 116.7, 117, 133, 149, 169, 172.
10a	14.14 (2C), 36.49, 48.74, 61.10, 61.96, 115.19, 116.74, 117.29, 127.65, 132.70, 148.88, 170.96, 171.17 (2C).

*6.1.2.1. N-(N-anthranoyl)anthranoyltryptamine (**24**).* R_f 0.53 (1:1 AcOEt–hexane); m.p. 169–170°C; ^1H NMR ($CDCl_3$): δ 3.07, t-2H (–C*H*$_2$–, indole); 3.69, q-2H (–NH–C*H*$_2$); 6.24, s-2H (–NH_2); 6.65–8.66, m-13H (arom.); 8.42, t-1H (–N*H*–CH_2–); 10.28, s-1H (–NH–, indole); 12.22, s-1H (–NH–). ^{13}C NMR ($CDCl_3$): δ 24.78, 40.26, 11.19, 111.87, 114.74, 115.78, 117.05, 118.14, 118.28, 120.44, 120.72, 120.96, 121.89, 122.13, 127.14, 127.31, 127.65, 131.49, 132.25, 136.23, 139.53, 149.97, 167.46, 168.84.

*6.1.2.2. N-(N-anthranoyl)anthranoyl-2-phenylethylamine (**25**).* R_f 0.69 (1:1 AcOEt–hexane); m.p. 158°C; ^1H NMR ($CDCl_3$): δ 2.93, t-2H (–C*H*$_2$–C_6H_5); 3.70, q-2H (–NH–C*H*$_2$–); 5.74, s-2H (–NH_2); 6.37, t-1H (–N*H*–CH_2); 6.67–8.62, m-13H (arom.); 11.74, s-1H (–NH–). ^{13}C NMR ($CDCl_3$): δ 35.55, 41.07, 115.73, 117.08, 117.45, 121.13, 121.75, 122.70, 126.46, 126.76, 127.88, 128.80, 132.36, 132.80, 138.56, 139.71, 149.74, 168.07, 169.14.

*6.1.3. General procedure for the preparation of compounds **11**–**20***

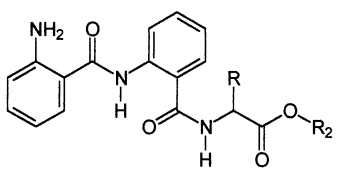
A mixture of 5 mmol of the corresponding ester (compounds **1**–**10**) in methanol (50 ml) and in the presence of potassium hydroxide (0.56 g, 10 mmol) was gently warmed for 4 h. The solvent was removed under reduced pressure and the residue taken up with water. After cooling, the solution was adjusted 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 sulphate and rotoevaporated to give the corresponding title compound. Crystallization from the appropriate solvent afforded the analytically pure derivatives **11**–**20** (Table 8).

Spectroscopic ^1H and ^{13}C NMR data are presented in Tables 9 and 10, respectively. The CCK-A receptor binding data of compounds **11**–**20** are summarized in Table 11.

*6.1.4. General procedure for the preparation of compounds **21**–**23***

Amides **21**–**23** were prepared from the corresponding esters (compounds **1**, **2** and **8**, respectively). A suspen-

Table 5
Physicochemical properties of compounds **1**–**10**



Comp.	R	R_2	Molecular formula	MW	Yield (%)	Crystal solvent [a]	R_f [b]	M.p. (°C)
1	–CH_2–3-indolyl	–C_2H_5	$C_{27}H_{26}N_4O_4$	470	69	A	0.45	150
2	–CH_2–C_6H_5	–C_2H_5	$C_{25}H_{25}N_3O_4$	431	71	A	0.73	137
3	–H	–C_2H_5	$C_{18}H_{19}N_3O_4$	341	36	B	0.51	128–129
4	–C_6H_5	–C_2H_5	$C_{24}H_{23}N_3O_4$	417	46	B	0.71	148–150
5	–$CH(CH_3)_2$	–C_2H_5	$C_{21}H_{25}N_3O_4$	383	32	B	0.63	118–120
6	–CH_2–$CH(CH_3)_2$	–C_2H_5	$C_{22}H_{27}N_3O_4$	397	48	C	0.66	95–96
7	–$(CH_2)_2$–CH_3	–CH_3	$C_{20}H_{23}N_3O_4$	369	18	B	0.64	118–119
8	–$(CH_2)_3$–CH_3	–CH_3	$C_{21}H_{25}N_3O_4$	383	56	B	0.70	113–114
9	–$(CH_2)_2$–S–CH_3	–C_2H_5	$C_{21}H_{25}N_3O_4S$	415	28	B	0.58	115–116
10	–CH_2–$COOC_2H_5$	–C_2H_5	$C_{22}H_{25}N_3O_6$	427	42	C	0.56	89–90

[a] Crystallizing solvents: A = EtOH 90%; B = MeOH; C = EtOH 50%.
[b] 1:1 AcOEt–hexane.

Table 6
^{1}H NMR ($CDCl_3$) of compounds **1–10**

Comp.	δ (ppm)
1	1.25, t-3H (–CH_3); 3.43, m-2H (–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 (arom.); 8.30, s-1H (–NH–, indole); 8.65, d-1H (arom.); 11.69, s–1H (–NH–).
2	1.28, t-3H (–CH_3); 3.23, m-2H (–CH_2–); 4.24, q-2H (–O–CH_2–); 5.03, m-1H (–CH<); 5.75, s-2H (–NH_2); 6.68–8.67, m-13H (arom.); 11.62, s-1H (–NH–).
3	1.29, t-3H (–CH_3); 4.17, d-2H (–CH_2–); 4.25, q-2H (–O–CH_2–); 5.74, s-2H (–NH_2); 6.66–6.77, m-2H (arom.); 6.95, t-1H (–N*H*–CH_2); 7.06–8.66, m-6H (arom.); 11.71, s-1H (–NH–).
4	1.24, t-3H (–CH_3); 4.24, q-2H (–O–CH_2–); 5.60, d-1H (–CH<); 5.75, s-2H (–NH_2); 6.66–8.76, m-14H (arom. and –N*H*–CH<); 11.60, s-1H (–NH–).
5	1.00, dd-6H (>CH–(CH_3)$_2$); 1.30, t-3H (–CH_2–CH_3); 2.27, m-1H (>C*H*–(CH_3)$_2$); 4.23, q-2H (–O–CH_2–); 4.75, m-1H (>C*H*–NH–); 5.75, s-2H (–NH_2); 6.66–6.77, m-2H (arom.); 6.82, d-1H (>CH–N*H*–); 7.08–8.68, m-6H (arom.); 11.63, s-1H (–NH–).
6	1.00, dd-6H (>CH–(CH_3)$_2$); 1.29, t-3H (–CH_2–CH_3); 1.73, m-3H (–CH_2–CH(CH_3)$_2$); 4.22, q-2H (–O–CH_2–); 4.83, m-1H (>C*H*–NH–); 5.75, s-2H (–NH_2); 6.67–8.68, m-9H (arom. and –N*H*–CH<); 11.65, s-1H (–NH–).
7	0.95, t-3H (–C*H*$_3$); 1.40, m-2H (–C*H*$_2$–CH_3); 1.82, m-2H (>CH–C*H*$_2$–); 3.77, s-3H (–O–CH_3); 4.81, m-1H (>C*H*–NH–); 5.75, s-2H (–NH_2); 6.67–6.77, m-2H (arom.); 6.81, d-1H (–N*H*–CH<); 7.07–8.68, m-6H (arom.); 11.66, s-1H (–NH–).
8	0.88, t-3H (–CH_3); 1.33, m-4H (–(CH_2)$_2$–CH_3); 1.81, m-2H (>CH–C*H*$_2$–); 3.78, s-3H (–O–CH_3); 4.80, m-1H (>C*H*–NH–); 5.75, s-2H (–NH_2); 6.66–6.77, m-2H (arom.); 6.81, d-1H (–N*H*–CH<); 7.07–8.68, m-6H (arom.); 11.66, s-1H (–NH–).
9	1.30, t-3H (–CH_3); 2.09, s-3H (–S–CH_3); 2.26, m-2H (>CH–C*H*$_2$–); 2.59, t-2H (–CH_2–S–); 4.24, q-2H (–O–CH_2); 4.89, m-1H (–CH<); 5.75, s-2H (–NH_2); 6.66–8.68, m-9H (arom. and –N*H*–CH<); 11.72, s-1H (–NH–).
10	1.25, t-3H (–CH_3); 1.28, t-3H (–CH_3); 3.00, m-2H (–CH_2–); 4.15, q-2H (–O–CH_2); 4.25, q-2H (–O–CH_2–); 5.02, m-1H (>CH–); 5.75, s-2H (–NH_2); 6.67–7.27, m-4H (arom.); 7.38, d-1H (–N*H*–CH<); 7.49–8.69, m-4H (arom.); 11.74, s-1H (–NH–).

Table 7
^{13}C NMR ($CDCl_3$) of compounds **1–10**

Comp.	δ (ppm)
1	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.
2	14.14, 37.97, 53.48, 61.83, 115.68, 116.92, 117.39, 120.29, 121.65, 122.71, 126.68, 127.28, 127.80, 128.60, 129.33, 132.73, 135.58, 139.93, 149.71, 167.94, 168.47, 171.22.
3	14.19, 41.85, 61.84, 115.70, 116.97, 117.47, 120.02, 121.64, 122.75, 126.97, 127.81, 132.83, 139.98, 149.74, 168.05, 169.19, 169.68.
4	14.06, 56.84, 62.26, 115.68, 116.91, 117.39, 120.13, 121.73, 122.64, 122.72, 126.95, 127.26, 127.91, 128.72, 128.99, 129.08, 132.74, 132.91, 136.25, 140.05, 149.73, 168.02, 168.42, 170.66.
5	14.26, 18.00, 18.99, 31.66, 57.38, 61.59, 115.70, 116.93, 117.44, 120.72, 121.75, 122.75, 126.78, 127.81, 132.75, 139.93, 149.77, 167.99, 168.97, 171.75.
6	14.20, 22.12, 22.89, 25.08, 41.77, 51.23, 61.64, 115.71, 116.90, 117.42, 120.53, 121.71, 122.71, 126.77, 127.86, 132.75, 139.96, 149.78, 168.01, 168.86, 172.81.
7	13.72, 18.66, 34.65, 52.40, 52.55, 115.71, 116.95, 117.44, 120.43, 121.75, 122.72, 126.81, 127.83, 132.76, 140.00, 149.77, 168.01, 168.79, 172.86.
8	13.88, 22.36, 27.40, 32.30, 52.54, 115.70, 116.96, 117.44, 120.46, 121.75, 122.74, 126.82, 127.83, 132.78, 139.98, 149.76, 168.01, 168.77, 172.85.
9	14.22, 15.06, 30.12, 31.45, 52.16, 62.00, 115.69, 116.93, 117.45, 120.13, 121.73, 122.76, 126.88, 127.81, 132.78, 132.92, 140.11, 149.78, 168.01, 168.90, 171.70.
10	14.14, 36.24, 48.97, 61.26, 62.18, 115.71, 116.91, 117.45, 119.98, 121.66, 122.77, 127.01, 127.78, 132.77, 132.96, 140.18, 149.79, 168.00, 168.70, 170.42, 171.12.

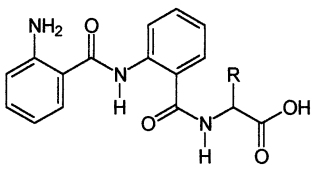
sion of 5 mmol of the corresponding ester in methanol (50 ml) was stirred and cooled at 0°C while a continuous stream of ammonia gas was bubbled into the reaction flask. After 2 h introduction of the ammonia was discontinued and the solution was allowed to warm up to r.t. and left to stand overnight. The solvent was removed in vacuo and the dry residue was crystallized from methanol to give the analytically pure target compounds.

*6.1.4.1. N-(N-anthranoyl)anthranoyltryptophanamide **(21)**.* R_f 0.23 (3:1 AcOEt–hexane); m.p. 248–249°C; ^{1}H NMR (DMSO-d_6): δ 3.26, m-2H (–CH_2–); 4.75, m-1H (–CH<); 6.50, s-2H (–NH_2); 6.66–8.53, m-13H (arom.); 7.69, s-2H (–CO–NH_2); 8.82, d-1H (–N*H*–CH<); 10.79, s-1H (–NH–, indole); 11.95, s-1H (–NH–). ^{13}C NMR (DMSO-d_6): δ 27.15, 53.91, 110.34, 111.14, 113.88, 115.02, 116.81, 118.04, 118.29, 120.03, 120.20, 120.71, 122.08, 123.40, 126.99, 127.40, 128.27, 131.91, 132.37, 135.87, 139.19, 150.19, 167.01, 168.38, 173.10.

*6.1.4.2. N-(N-anthranoyl)anthranoylphenylalaninamide **(22)**.* R_f 0.54 (3:1 AcOEt–hexane); m.p. 256°C; ^{1}H NMR (DMSO-d_6): δ 3.15, m-2H (–CH_2–); 4.73, m-1H (–CH<); 6.58, s-2H (–NH_2); 6.63–8.52, m-13H (arom.); 7.68, s-2H (–CO–NH_2); 8.91, d-1H (–N*H*–CH<); 11.79, s-1H (–NH–). ^{13}C NMR (DMSO-d_6): δ 36.96, 54.40, 113.84, 114.93, 116.80, 120.01, 120.27, 122.09, 126.03, 127.02, 127.80, 128.23, 128.88, 131.83, 132.37, 138.18, 139.04, 150.24, 166.95, 168.35, 172.60.

*6.1.4.3. N-(N-anthranoyl)anthranoylnorleucinamide **(23)**.* R_f 0.44 (3:1 AcOEt–hexane); m.p. 210–211°C; ^{1}H NMR (DMSO-d_6): δ 0.85, t-3H (–CH_3); 1.31, m-4H

Table 8
Physicochemical properties of compounds **11–20**



Comp.	R	Molecular formula	MW	Crystal solvent [a]	R_f	M.p. (°C)
11	–CH_2–3-indolyl	$C_{25}H_{22}N_4O_4$	442	A	0.26 [b]	217–219
12	–CH_2–C_6H_5	$C_{23}H_{21}N_3O_4$	403	A	0.54 [b]	176–177
13	–H	$C_{16}H_{15}N_3O_4$	313	A	0.34 [c]	198–199
14	–C_6H_5	$C_{22}H_{19}N_3O_4$	389	A	0.52 [c]	244
15	–$CH(CH_3)_2$	$C_{19}H_{21}N_3O_4$	355	B	0.65 [c]	199
16	–CH_2–$CH(CH_3)_2$	$C_{20}H_{23}N_3O_4$	369	B	0.69 [c]	177
17	–$(CH_2)_2$–CH_3	$C_{19}H_{21}N_3O_4$	355	A	0.66 [b]	211
18	–$(CH_2)_3$–CH_3	$C_{20}H_{23}N_3O_4$	369	A	0.52 [b]	204
19	–$(CH_2)_2$–S–CH_3	$C_{19}H_{21}N_3O_4S$	387	A	0.37 [b]	210–211
20	–CH_2–COOH	$C_{18}H_{17}N_3O_6$	371	A	0.63 [d]	210

[a] Crystallizing solvents: A = MeOH; B = EtOH 95%.
[b] 3:1 AcOEt–MeOH.
[c] 2:1 AcOEt–MeOH.
[d] 1:2 AcOEt–MeOH.

Table 9
^{1}H NMR (DMSO-d_6) of compounds **11–20**

Comp.	δ (ppm)
11	3.36, m-2H (–CH_2–); 4.75, m-1H (>CH–); 6.53–8.65, m-15H (arom. and –NH_2); 9.04, d-1H (–N*H*–CH<); 10.83, s-1H (–NH–, indole); 11.95, s-1H (–NH–).
12	3.21, m-2H (–CH_2–); 4.70, m-1H (>CH–); 6.57–8.56, m-15H (arom. and –NH_2); 9.10, d-1H (–N*H*–CH<); 11.80, s-1H (–NH–).
13	3.97, d-2H (–CH_2–); 6.59–8.62, m-10H (arom. and –NH_2); 9.22, t-1H (–N*H*–CH_2–); 12.15, s-1H (–NH–); 12.80, b-1H (–OH).
14	5.62, d-1H (–CH<); 6.58–8.52, m-13H (arom. and –NH_2); 9.40, d-1H (–N*H*–CH<); 11.78, s-1H (–NH–).
15	1.00, d-6H (–CH–(CH_3)$_2$); 2.23, m-1H (>C*H*–(CH_3)$_2$); 4.31, m-1H (–NH–C*H*<); 6.58–8.53, m-10H (arom. and –NH_2); 8.83, d-1H (–N*H*–CH<); 11.76, s-1H (–NH–); 12.80, b-1H (–OH).
16	0.91, d-6H (–CH–(CH_3)$_2$); 1.75, m-2H (–CH_2–); 4.50, m-1H (–NH–C*H*<); 6.58–8.57, m-10H (arom. and –NH_2); 8.99, d-1H (–N*H*–CH<); 11.94, s-1H (–NH–); 12.75, b-1H (–OH).
17	0.90, t-3H (–CH_3); 1.41, m-2H (–CH_2–CH_3); 1.80, m-2H (>CH–CH_2–); 4.40, m-1H (>CH–); 6.59–8.55, m-10H (arom. and –NH_2); 8.97, d-1H (–N*H*–CH<); 11.91, s-1H (–NH–).
18	0.87, t-3H (–CH_3); 1.35, m-4H ((–CH_2)–CH_3); 1.83, m-2H (>CH–CH_2–); 4.40, m-1H (>CH–); 6.59–8.57, m-10H (arom. and –NH_2); 8.98, d-1H (–N*H*–CH<); 11.93, s-1H (–NH–).
19	2.06, s-3H (–CH_3); 2.11, m-2H (>CH–CH_2); 2.62, m-2H (–S–CH_3); 4.57, m-1H (>CH–); 6.59–8.54, m-10H (arom. and –NH_2); 9.03, d-1H (–N*H*–CH<); 11.93, s-1H (–NH–).
20	2.84, m-2H (–CH_2); 4.79, m-1H (>CH–); 6.60–8.60, m-10H (arom. and –NH_2); 9.12, d-1H (–N*H*–CH<); 12.00, s-1H (–NH–); 12.70, b-2H (–OH).

((–CH_2)$_2$–CH_3); 1.77, m-2H (>CH–CH_2–); 4.39, m-1H (>CH–); 6.58, s-2H (–NH_2); 6.65–8.54, m-8H (arom.); 7.07, s-2H (–CO–NH_2); 8.73, d-1H (–N*H*–CH<); 11.96, s-1H (–NH–). ^{13}C NMR (DMSO-d_6): δ 13.67, 21.64, 27.79, 30.97, 53.08, 113.83, 114.95, 116.84, 120.27, 120.69, 122.24, 127.03, 128.50, 131.79, 132.39, 139.01, 150.28, 167.04, 168.49, 173.30.

6.2. Biochemistry

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 (Bruxelles) 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 previously described [22]. Radioactivity was counted with 4 ml of Aquassure (NEN) high performance LSC cocktail in a Packard TRI-CARB 300 liquid scintillator.

6.2.2. Radioligand binding assays

All experiments were performed in triplicate.

CCK-A receptor affinities were determined by displacement of [^{3}H]-(±)-L-364,718 from rat pancreas membranes as previously described [19]. 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

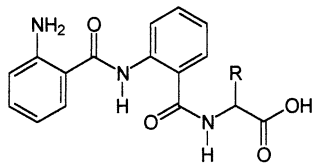
Table 10
^{13}C NMR (DMSO-d_6) of compounds **11**–**20**

Comp.	δ (ppm)
11	26.28, 53.64, 110.13, 111.31, 113.77, 115.09, 116.91, 117.96, 118.25, 119.68, 120.06, 120.83, 122.16, 123.44, 126.98, 128.31, 132.15, 132.49, 135.97, 139.35, 150.36, 167.04, 168.69, 172.88.
12	36.00, 53.89, 113.71, 115.02, 116.85, 119.84, 120.05, 122.17, 126.19, 126.95, 127.94, 128.10, 128.83, 132.03, 132.45, 137.78, 139.13, 150.30, 166.95, 168.51, 172.49.
13	41.08, 113.77, 115.02, 116.87, 119.43, 120.17, 122.26, 126.97, 127.97, 132.18, 132.45, 139.47, 150.31, 167.05, 168.90, 170.63.
14	56.71, 113.75, 115.01, 116.85, 120.27, 120.34, 122.27, 127.01, 127.82, 128.04, 128.22, 128.85, 132.06, 132.45, 136.27, 138.98, 150.28, 167.03, 168.42, 171.31.
15	18.51, 19.06, 29.15, 58.21, 113.62, 114.99, 116.87, 120.26, 120.58, 122.26, 126.93, 128.72, 131.91, 132.45, 138.84, 150.30, 166.86, 168.93, 172.43.
16	20.92, 22.76, 24.42, 38.95, 50.76, 113.71, 115.00, 116.89, 120.18, 120.23, 122.25, 126.95, 128.38, 132.01, 132.46, 139.10, 150.34, 167.02, 168.77, 173.51.
17	13.25, 18.88, 32.15, 52.25, 113.77, 115.09, 116.88, 120.28, 120.42, 122.33, 126.96, 128.39, 132.01, 132.49, 138.99, 150.24, 167.03, 168.83, 173.23.
18	13.58, 21.53, 27.80, 29.87, 52.47, 113.74, 115.03, 116.88, 120.27, 120.50, 122.28, 126.96, 128.41, 131.99, 132.46, 139.05, 150.30, 167.03, 168.81, 173.20.
19	14.36, 29.75, 29.90, 51.47, 113.72, 115.02, 116.87, 120.07, 120.20, 122.25, 126.97, 128.41, 132.08, 132.46, 139.12, 150.31, 167.02, 168.95, 172.82.
20	34.69, 48.51, 113.10, 114.44, 116.26, 119.03, 119.54, 121.60, 126.35, 127.53, 131.56, 131.85, 138.69, 149.70, 166.40, 167.78, 170.84, 171.28.

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.

Central CCK-B receptor affinities were determined by displacement of [^{3}H]-(+)-L-365,260 from guinea pig cerebral cortex membranes as previously described by Chang et al. [20]. 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 compound 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.

Table 11
CCK receptors binding data



Comp.	R	CCK-A (IC_{50}) [a]
11	–CH_2–3-indolyl	7.0
12	–CH_2–C_6H_5	26
13	–H	IN [c]
14	–C_6H_5	24 [b]
15	–$CH(CH_3)_2$	IN [c]
16	–CH_2–$CH(CH_3)_2$	30 [b]
17	–$(CH_2)_2$–CH_3	26
18	–$(CH_2)_3$–CH_3	24
19	–$(CH_2)_2$–S–CH_3	22
20	–CH_2–COOH	IN [c]

[a] 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.

[b] Percentage of inhibition at 30 μM of [^{3}H]-(±)-L-364,718 binding in rat pancreatic membranes.

[c] Inactive: the test compound inhibited [^{3}H]-(±)-L-364,718 specific binding in rat pancreatic membranes by less than 20% at 30 μM.

References

[1] J.A. Williams, Biomed. Res. 3 (1982) 107–121.
[2] J.N. Crawley, R.L. Corwin, Peptides 15 (1994) 731–755.
[3] R.B. Innis, S.H. Snyder, Proc. Natl. Acad. Sci. USA 77 (1980) 6917–6921.
[4] C.T. Dourish, D.R. Hill, Trends Pharmacol. Sci. 8 (1987) 207–208.
[5] M. D'Amato, F. Makovec, L.C. Rovati, Drug News Perspect. 7 (1994) 87–95.
[6] D.R. Hill, L. Singh, P. Boden, R. Pinnock, G.N. Woodruff, J. Hughes, in: C.T. Dourish, S.J. Cooper, S.D. Iversen, L.L. Iversen (Eds.), Multiple Cholecystokinin Receptors in the CNS, Oxford University Press, London, 1992, pp. 57–76.
[7] A. Rex, C.A. Marsden, H. Fink, Neurosci. Lett. 228 (1997) 79–82.
[8] S. Ballaz, A. Barber, A. Fortuno, J. Del Rio, M. Martin-Martinez, I. Gomez-Monterrey, R. Herranz, R. Gonzalez-Muniz, M.T. Garcia-Lopez, Br. J. Pharmacol. 121 (1997) 759–767.
[9] L. Singh, R.J. Oles, M.J. Field, P. Atwal, G.N. Woodruff, J.C. Hunter, Br. J. Pharmacol. 118 (1996) 1317–1325.
[10] M.O. Urban, D.J. Smith, G.F. Gebhart, J. Pharmacol. Exp. Ther. 278 (1996) 90–96.
[11] F. Makovec, Drugs Future 18 (1993) 919–931.
[12] F. Makovec, W. Peris, S. Frigerio, R. Giovanetti, O. Letari, L. Mennuni, L. Revel, J. Med. Chem. 39 (1996) 135–142.
[13] 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, Proc. Natl. Acad. Sci. USA 83 (1986) 4918–4922.

[14] 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, J. Med. Chem. 32 (1989) 13–16.
[15] M.J. Yu, K.J. Trasher, J.R. McCowan, N.R. Mason, L.G. Mendelson, J. Med. Chem. 34 (1991) 1505–1508.
[16] P.R. Boden, M. Higginbottom, D.R. Hill, D.C. Horwell, J. Hughes, D.C. Rees, E. Roberts, L. Singh, N. Suman-Chauhan, G.N. Woodruff, J. Med. Chem. 36 (1993) 552–565.
[17] M.J. Drysdale, M.C. Pritchard, D.C. Horwell, J. Med. Chem. 35 (1992) 2573–2581.
[18] A. Varnavas, L. Lassiani, E. Luxich, M. Zacchigna, Pharmazie 51 (1996) 697–700.
[19] R.S.L. Chang, V.J. Lotti, T.B. Chen, K.A. Kunkel, Mol. Pharmacol. 30 (1986) 212–217.
[20] R.S.L. Chang, T.B. Chen, M.G. Bock, R.M. Freidinger, R. Chen, A. Rosegay, V.J. Lotti, Mol. Pharmacol. 35 (1989) 803–808.
[21] 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, J. Med. Chem. 29 (1986) 1941–1945.
[22] M.G. Bock, R.M. Di Pardo, B.E. Evans, K.E. Rittle, D.F. Veber, R.M. Freidinger, J. Hirshfield, J.P. Springer, J. Org. Chem. 52 (1987) 3232–3239.