N-Terminal Anthranoyl-Phenylalanine Derivatives as CCK_1 Receptor Antagonists: The Final Approach

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Abstract: Starting from our lead compound, **VL-0395**, an anthranilic acid based CCK_1 receptor antagonist, and following the well established "step by step" lead investigation strategy, we describe the final step of the anthranilic acid N-terminal optimization. Improvements for both affinity and selectivity towards CCK_1 receptors have been accomplished through introduction of the fluoro substituent at C-5 and C-7 position of the indole ring together with the appropriate configuration of the aminoacidic chiral center.

Key Words: Cholecystokinin, CCK, Receptors, CCK1, Anthranilic Acid, Phenylalanine derivatives, Indole, Antagonists, Needle, Ligands.

INTRODUCTION

Cholecystokinin (CCK) is a peptide hormone involved in numerous physiological processes throughout the gastrointestinal (GI) tract and the central nervous system (CNS) [1-2]. The multiple biological profiles of CCK are mediated by two receptor subtypes CCK_1 and CCK_2 which are both members of the seven transmembrane domain G-protein-coupled receptor (GPCR) superfamily [3-4]. The CCK_1 receptor is mainly located in peripheral tissues such as gallbladder and pancreas and exhibits a higher affinity for the sulphated CCK octapeptide (CCK-8S) than for the nonsulphated form (CCK-8) and the C-terminal tetrapeptide (CCK-4) [5-6]. Conversely, CCK_2 receptors are located mainly in the CNS and bind with nanomolar affinity both CCK-8 and CCK-4 while poorly discriminate between the sulphated and nonsulphated CCK-8 forms [7-9].

In the last decade there has been a great impetus to develop small non-peptide molecules known as CCK receptor antagonists [10-12]. For each of these classes, some compounds that exhibit almost subnanomolar and selective affinity have been described. They present high structural dissimilarities that could be considered responsible for the different ADMET properties (absorption, distribution, metabolism, excretion and toxicity) and for more or less complexity in their synthesis. Obviously, in choosing a promising candidate as a lead or for clinical development, all these parameters must be taken into account.

It is thought that CCK antagonists can be used with advantage in the therapy of various pathologies tied to lack of balance of CCK or other related bioactive peptides at peripheral level (GI tract) as well as at the level of the CNS. In particular, at the GI tract they can be used for the treatment of biliary colic in case of cholecystitis or cholelythiasis, in the gastro-esophageal reflux (GERD) due to an anomalous functioning of the lower esophageal sphincter (LES) as well as in irritable bowel syndrome (IBS).

Among the strategies adopted for the design of these antagonists, a particular consideration is given to chemical simplification and manipulation of natural products, such as asperlicin [13,14], the dipeptoid approach, in which the minimal structure responsible for the binding is a dipeptide derived from the C-terminal sequence of CCK [15-16], and the key aminoacid derivatization, in which the receptor recognition is assigned to a single aminoacid [17]. All these strategies include a final optimization step of the obtained lead compound, performed by chemical modifications.

As a result of our interpretation and integration of these approaches, we described in an earlier report the discovery of a new class of CCK_1 receptor ligands, characterized by the presence of two pharmacophores selected from the C-terminal tetrapeptide of CCK and by the anthranilic acid dimer used as a molecular scaffold [18-19]. The lead compound **VL-0494** Fig. (**1**) obtained in this part of our work contains some of the structural features of CCK-4 with both molecules having the same N- and C-terminal aminoacid side chains but differs in the presence of the anthranilic acid dimer instead of the Met-Asp central dipeptide respectively [19]. Moreover, although **VL-0494** and CCK-4 have a reversal selectivity for the CCK-receptors, the micromolar affinity of **VL-0494** toward CCK_1 receptors has been ascribed to the conformational ability of the anthranilic acid dimer to mimic the regnylogical bioactive organization of CCK-4 [20-21].

Further simplification of the anthranilic acid dimer scaffold to a monomer led to compound **VL-0395** Fig. (**1**), endowed with sub-micromolar affinity (IC_{50} = 0.197 μM) that represented a new starting point for the development of this innovative class of CCK_1 receptor antagonists [22]. The antagonistic nature of **VL-0395** was confirmed by an *in vivo* functional test. The compound, intravenously administered, inhibited the guinea pig gallbladder contractions induced by CCK8 sulphated (8.8 pmol/kg i.v.), with an ED_{50} of 0.38 μmol/kg (0.11-1.36; p = 0.05 fiducial limits). Its potency was

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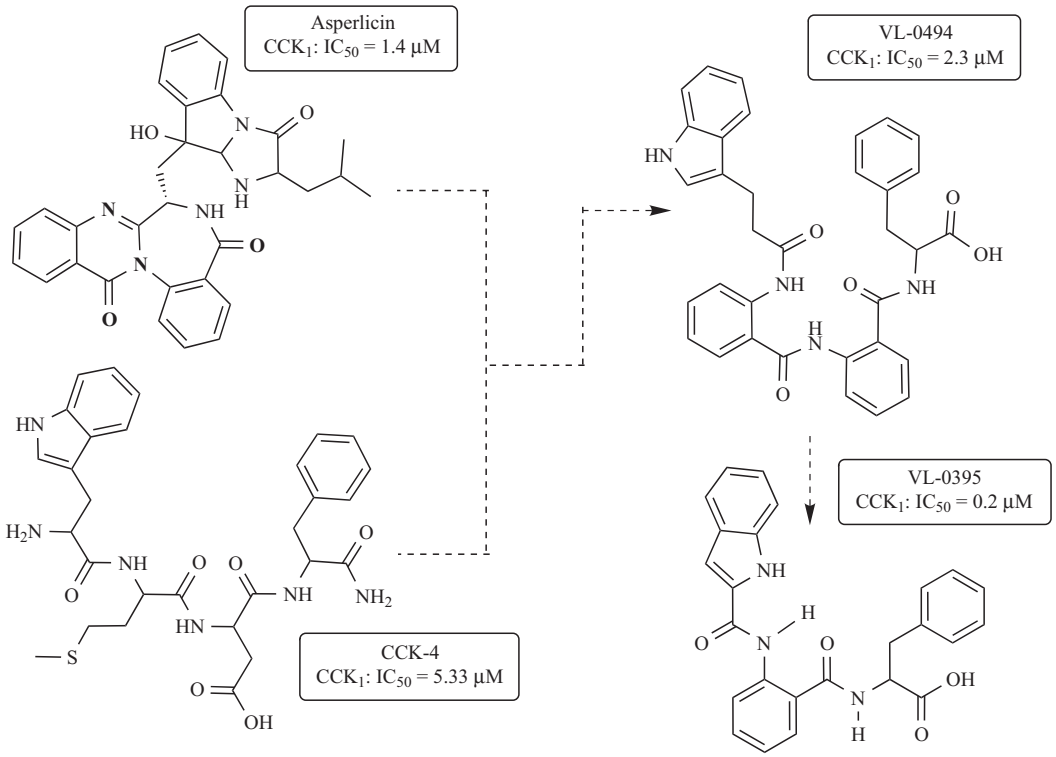


Fig. (1).

comparable with that exhibited by the reference CCK_1 selective antagonist, Loxiglumide (n. 5610 Merck Index, 13th Edition) [23], that was active, under the same experimental conditions, at 0.24 μmol/kg (0.13-0.41; p = 0.05 fiducial limits).

Moreover, on the basis of the main pharmacophoric components shared by **VL-0395** (indole moiety and phenyl ring of Phe), a receptor binding hypothesis, similar to that described for the anthranilic acid dimer derivatives, has been proposed. In fact, this model of binding involves at least two hydrophobic pockets in order to accommodate the main pharmacophoric groups [22].

Furthermore, in the first optimizational step of the pharmacophoric group linked at the N-terminal site of the anthranilic acid, we found that the replacement of the 2-indole moiety by other indole isomers as well as by aliphatic, aromatic, bi-aromatic rings or by heterocycles lacking the fused phenyl ring caused, to a greater or lesser extent, a decrease in binding affinity [24]. All these findings suggest that this receptor binding sub-site imposes a high degree of conformational restrictions. Since the 2-indole group is the unique moiety able to establish very specific interactions with the receptor we have hypothesized that it may be viewed as a "needle" type pharmacophoric group in this new class of CCK_1 receptor antagonists.

Thus, to further explore the receptor sub-site topography and its tolerance at the 2-indole moiety, we synthesized and tested the N-terminal anthranilic acid derivatives reported in Table **1**. These compounds are characterized by the presence of substituted 2-indole groups at the N-terminus of the anthranilic acid.

First, we studied the binding effect of the substitution in position 5 of the indole ring (a preferential position for the most naturally occurring indoles) (compounds **1-8**).

Then, the substituent which confers the higher affinity has been utilized as a probe in order to explore the topographic tolerance of the receptor binding sub-site by its introduction to different positions of the indole ring (compounds **9-15**).

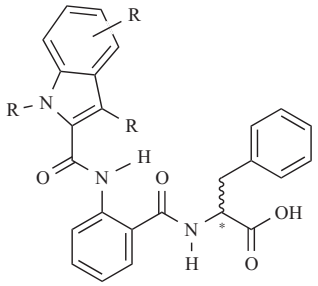
In the final step, we extended the study on the binding effect in relation to the receptor subsite stereospecificity, determining the eutomer and the eudismic ratio (ER) of the two pure enantiomers of the lead **VL-0395** and of the other anthranilic acid derivatives endowed with significant affinity for the CCK_1 receptors (compounds **16-21**).

CHEMISTRY

As indicated in Scheme 1, the new anthranoyl-phenyl-alanine derivatives (compounds **1-21)** were prepared applying the synthetic route used for the lead compound **VL-0395** [22].

In general, this route involved reaction of isatoic anhydride with the corresponding DL, L or D-phenylalanine ethyl ester to give the N-anthranoyl derivatives **22-24** respectively. Then, compounds **1c-21c** were obtained from the corresponding acids *via* acyl chloride formation by a standard method using PCl_5 in dry dichloromethane. The indole 2-carboxylic acids employed for compounds **1c-8c** were commercially available while all the others (compounds **9a-15a**) were prepared according to two different synthetic pathways.

Table 1. Structure of the Target Compounds

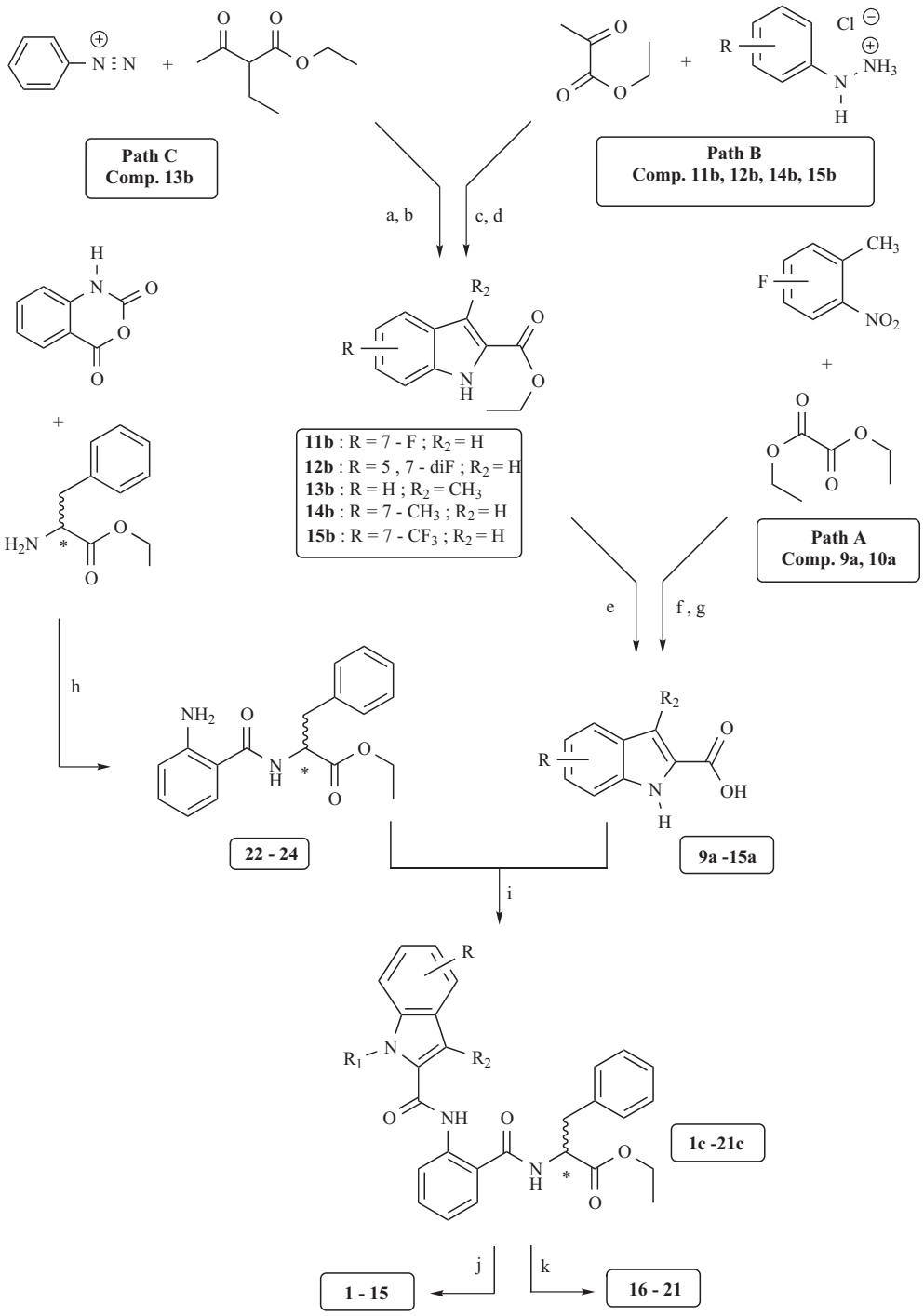


Comp.	R	R_1	R_2	STEREO
1	5-Methyl	H	H	R,S
2	5-Methoxy	H	H	R,S
3	5,6-Dimethoxy	H	H	R,S
4	5-Benzyloxy	H	H	R,S
5	5-Chloro	H	H	R,S
6	5-Nitro	H	H	R,S
7	5-Nitro	CH_3	H	R,S
8	5-Fluoro	H	H	R,S
9	4-Fluoro	H	H	R,S
10	6-Fluoro	H	H	R,S
11	7-Fluoro	H	H	R,S
12	5,7-Difluoro	H	H	R,S
13	H	H	CH_3	R,S
14	7-Methyl	H	H	R,S
15	7-Trifluoromethyl	H	H	R,S
16	H	H	H	S
17	H	H	H	R
18	5-Fluoro	H	H	S
19	5-Fluoro	H	H	R
20	7-Fluoro	H	H	S
21	7-Fluoro	H	H	R

The 4- and 6-fluoro substituted indole-2-carboxylic acids **9a** and **10a** were obtained by the Reissert method (**Path A**). In particular the *o*-nitro-phenylpyruvic intermediate, synthesized starting from ethyl oxalate and the appropriately substituted fluoronitrotoluene [25,26], was reduced by treatment with ferrous sulphate and ammonia as described by Kermack [27].

The acids **11a-15a** were obtained by Fischer indolization, catalyzed by acidic conditions, of the phenylhydrazones intermediate. These were prepared by two different methods. For compound **13b** the hydrazone was synthesized as described by Kitano *et al*. [28], starting from ethyl 2-ethylacetacetate and diazotized aniline, followed by Japp-Kligemann reaction and cyclization catalyzed by HCl in EtOH (**Path C**).

For compounds **11b**, **12b**, **14b** and **15b**, (**Path B**) the phenylhydrazones were prepared from the corresponding substituted hydrazines and ethyl pyruvate. In these cases the indolization was carried out with $ZnCl_2$/AcOH [29] which seems to be the better choice to increase the yield [30]. The ethyl esters **11b-15b** were then converted into the corresponding acids **11a-15a** by hydrolysis as described by Murakami [29].



Scheme (1). Reagents and reaction conditions: (a) KOH 50%, EtOH, 0 °C; (b) HCl (g), EtOH abs., reflux 15 min; (c) CH_3COONa, H_2O, MeOH; (d) $ZnCl_2$, AcOH, reflux; (e) KOH, EtOH, reflux; (f) NaOEt, EtOH abs., reflux; (g) $FeSO_4 \cdot 7H_2O$, NH_4OH 32%, H_2O, reflux 1h; (h) Et_3N, AcOEt, reflux 5h; (i) RCOOH, PCl_5, CH_2Cl_2 dry, pyridine; (j) NaOH or KOH, THF/H_2O 1:1; (k) LiOH•H_2O, THF/H_2O 1:1.

The optically active compounds **16-21** were obtained by base catalyzed hydrolysis with LiOH of the corresponding ethyl esters **16c-21c**. In a similar manner, using an aqueous solution of NaOH or KOH, the final compounds **1-15** were obtained as racemates from the esters **1c-15c**.

The optical purity of the enantiomers (compounds **16-21**), obtained by the above depicted syntheses starting from D- or L-aminoacids, was determined by analytical enantioselective HPLC (EHPLC) under reverse phase conditions. The employed chiral stationary phase (CSP) contains native teicoplanin chemically bonded according to a recently published procedure [31].

Different detectors were used, including chiro-optical (circular dichroism) and UV detectors as well as evaporative

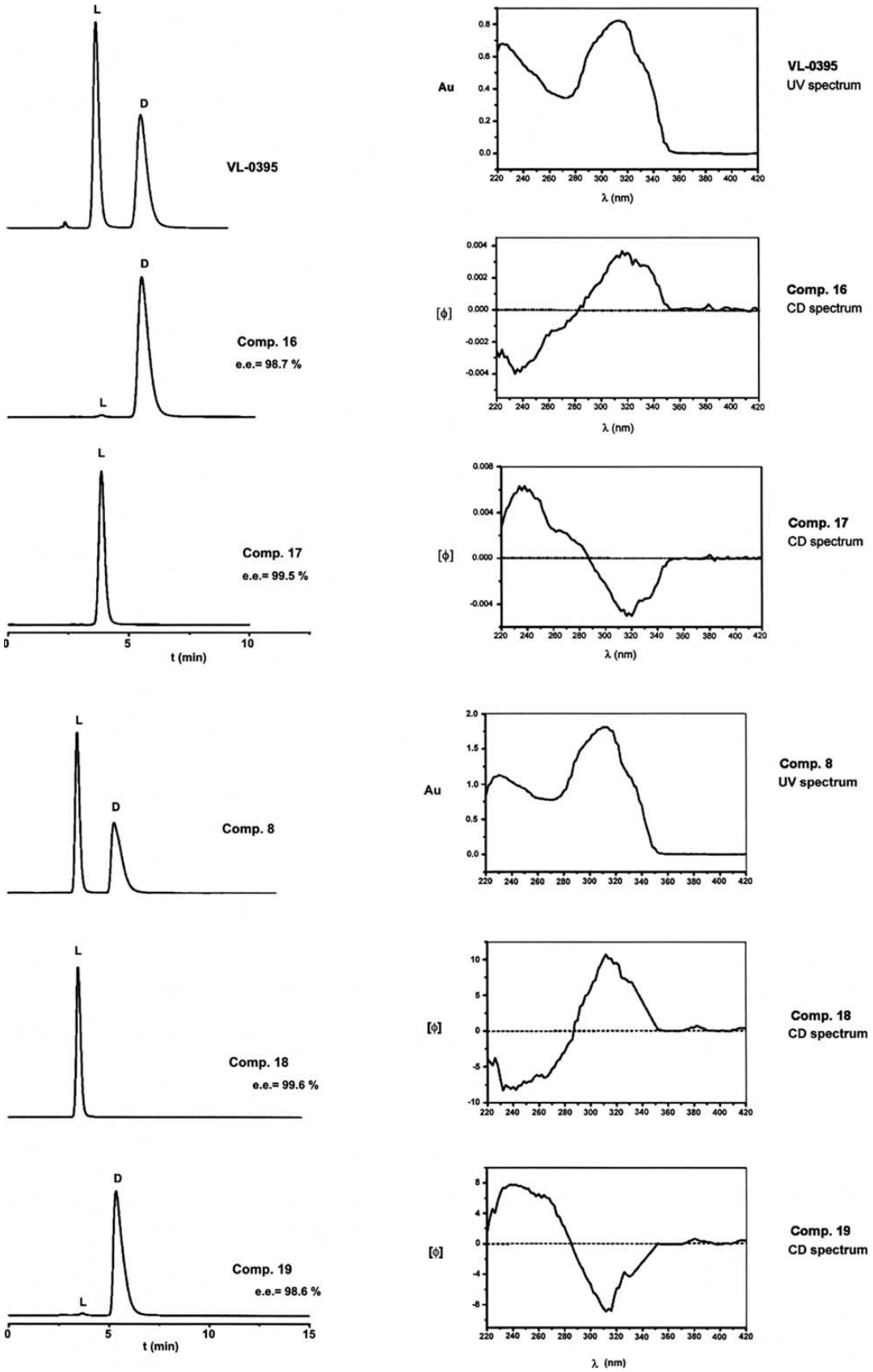


Fig. (2). Chromatograms, UV and CD spectra of the racemic **VL-0395** and **8** compounds and of the corresponding pure enantiomers (compounds **16-19**).

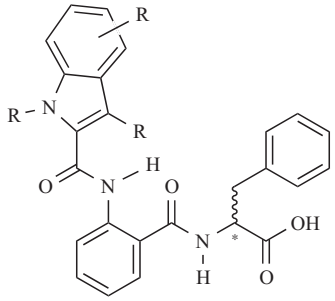
light-scattering detection (ELSD). Example of the obtained chromatograms along with UV and CD spectra of racemic compounds **VL-0395** and **8** and the corresponding pure enantiomers (compounds **16**-**19**) are reported in Fig. (**2**).

RESULTS AND DISCUSSION

The binding affinities for CCK_1 and CCK_2 receptors of the synthesized compounds **1-21** were evaluated according to established protocols [17] and are expressed as IC_{50} or as percentage of inhibition (ISB %) determined at the highest used dose (1 μM, 3 μM or 30 μM as indicated). Values without standard errors were obtained from no more than two experiments.

Binding data, selectivity indices (SI, expressed as CCK_2/CCK_1 ratio) and the eudismic ratio for the optically active compounds, along with those of the lead **VL-0395** (Comp. **0**), all useful for the following discussion of SAfR, are reported in Table **2**.

Table 2. CCK Receptors Binding Data



					IC_{50}[a] (μM)			
Comp.	**R**	**R_1**	**R_2**	**Stereo**	**Rat pancreatic acini (CCK_1)**	**Guinea pig brain cortex (CCK_2[f])**	**SI (CCK_2/CCK_1)**	**ER**
0	H	H	H	R,S	0.197± 0.107	16.40		
1	5-Methyl	H	H	R,S	2.791 ± 0.582	4.2		
2	5-Methoxy	H	H	R,S	42% ISB [c]	17.1		
3	5,6-Dimethoxy	H	H	R,S	IN[b]	IN[c]		
4	5-Benzyloxy	H	H	R,S	IN[b]	IN[c]		
5	5-Chloro	H	H	R,S	1.841 ± 0.189	31% ISB[e]		
6	5-Nitro	H	H	R,S	3.550	49 % ISB[e]		
7	5-Nitro	CH_3	H	R,S	1.108 ± 0.189	IN[c]		
8	5-Fluoro	H	H	R,S	0.314 ± 0.016	10.6		
9	4-Fluoro	H	H	R,S	0.937 ± 0.097	9.6		
10	6-Fluoro	H	H	R,S	0.294 ± 0.015	36.8		
11	7-Fluoro	H	H	R,S	0.159 ± 0.025	18.7		
12	5,7-Difluoro	H	H	R,S	59% ISB[d]	9.4		
13	H	H	CH_3	R,S	2.057 ± 0.263	9.8		
14	7-Methyl	H	H	R,S	0.671 ± 0.101	10.2		
15	7-Trifluoromethyl	H	H	R,S	0.538 ± 0.064	11.2		
16	H	H	H	S	10.2 ± 0.5	52 % ISB[e]	--	96
17	H	H	H	R	0.106 ± 0.014	17.0 ± 2.3	160	
18	5-Fluoro	H	H	S	30.1 ± 1.5	20 % ISB[e]	--	199
19	5-Fluoro	H	H	R	0.151 ± 0.009	67.9 ± 3.6	450	
20	7-Fluoro	H	H	S	3.55 ± 0.15	8.9 ± 1.5	--	65
21	7-Fluoro	H	H	R	0.055 ± 0.006	19.8 ± 1.4	360	

[a] IC_{50} ± standard error (ALLFIT analysis); % ISB: percentage inhibition of specific binding of 25 pM [^{125}I]-(BH)-CCK8 at the maximal concentration tested, [b] 1 μM, [c] 3 μM, [d] 10 μM, and [e] 30 μM. [f] Values without standard errors were obtained from not more than two experiments; IN: inactive.

The present compounds can be divided into three groups (Table **2**: bold lines) and will be discussed separately according to the prefixed targets and their step-wise development based on the affinity obtained in the previous step.

Like previous affinity data reported on the anthranilic acid derivatives [22,24], no appreciable affinity was observed toward CCK_2 receptors. Nevertheless, it is interesting to notice that compound **1** showed comparable micromolar affinity for both CCK receptors suggesting the possibility to obtain mixed type antagonists.

With respect to their affinity for CCK_1 receptors, compounds (**1-8**), bearing substituents at C-5 of the indole ring with differing electronic and steric properties, revealed a lower affinity than that of the lead compound **VL-0395** (Comp. **0**). Although the limited set of compounds, a macroscopic analysis of the obtained data shows that the binding affinity of these ligands appears clearly influenced by the steric effect rather than by electronic parameters. In fact, compounds **1** and **5** characterized by the presence of substituents with comparable steric hindrance but with opposite electronic effects are endowed with similar binding affinity. Indeed, higher binding affinity was observed for substituents with a minor steric hindrance among compounds with the same type of electronic effect (Comp. **5** *vs* Comp. **8**).

The detrimental effect on the affinity showed by the bulkier groups, as in compound **2** and especially in compound **4**, could be ascribed to a steric clash with adjacent residues that weakens the interaction with the "indole" receptor subsite.

It is interesting to notice that among the herein reported compounds, starting from commercially available indoles (comp. **1-8**), compound **8** possesses the highest affinity, comparable to that of **VL-0395**.

The fact that the presence of a 5-fluoro substituent on the indole ring doesn't disrupt the receptor binding (comp. **8**), while bulkier substituents (i.e. Cl or CH_3) decrease significantly the affinity, confirms the important role of the steric factors for the correct anchoring of the anthranilic acid N-terminal pharmacophore and strengthens our previous results on the strict steric requirements of this receptor subsite [24].

Hence, taking into account that the 5-fluoro substituent, probably thanks to its reduced dimensions, shows a comparable affinity to that of the lead, we decided to synthesize compounds **9-11.**

These compounds bear the fluoro substituent at the other positions of the indole benzo-fused ring and allow the exploration of the spatial limitations of the receptor recognition site which interacts with this part of the molecule.

As seen from the examination of the results listed in Table 2, the position of the fluoro substituent produces quite different effects. So, while the presence of the fluoro "probe" at C-6 and C-7 (compounds **10** and **11**) appears to be well tolerated, the lower affinity exhibited by compound **9** seems to indicate a preferential space interaction of this pharmacophore with its receptor pocket. In fact, the hydrophobic receptor subsite offers an asymmetric space to the indole binding conformation. This can be viewed considering the equipotent compounds **8** and **10** in contrast to the six fold more potent compound **11** with respect to the anti-diametric C-4 derivative (comp. **9**).

Moreover, the difluoro derivative (compound **12**) was synthesized in order to explore a possible additive binding effect by the simultaneous presence of the two fluorine atoms on the indole benzo-fused ring. However, a deletion of the binding improvement obtained with the most active compound **11** was observed for compound **12**, having the fluoro substituent in favorable positions such as C-7 and C-5.

Subsequently a methyl group was used as receptor subsite spatial probe relative to the C-3 of the indole ring (compound **13**) because of the lack in the chemical literature of a suitable synthetic procedure for the preparation of the 3-fluoro 2-indole carboxylic acid. Moreover the presence of the methyl group on the pyrrole ring was preferred, over other substituents, for the comparison with the N-methyl indole derivative which was shown to be equipotent to the lead **VL-0395** [24].

A negative binding effect was observed even for compound **13** bearing a methyl group at C-3 position of the indole moiety. This remarkable decrease in affinity confirms the spatial receptor restrictions for this side of the indole nucleus.

The above-mentioned findings are useful to better elucidate our previously reported receptor binding model relative to the indole pharmacophoric group. In fact, we can now hypothesize that in the indole interaction approach, the C-5 and C-6 carbon atoms are directed toward the bottom of the hydrophobic pocket (comparable affinities of compounds **8** and **10**), so leaving more space at the N-indole side with respect to the opposite one as indicated by the higher affinity of compound **11** with respect to the indole C-3 or C-4 derivatives (compounds **13** and **9** respectively).

Encouraged by the promising result of compound **11**, we next forced the receptor space tolerance relative to the indole C-7 position by introducing the bulkier methyl and trifluoromethyl groups (compounds **14** and **15** respectively). Nevertheless, the bulkier groups at C-7 lead to a drop in affinity suggesting that this indole position could advantageously support only the smaller fluoro substituent.

Finally, having successfully obtained the fine-tuning of the indole receptor interaction, compounds **16**-**21** were synthesized in order to investigate and validate the influence of the stereochemistry of **VL-0395** and that of two other active compounds of this series (**8** and **11**) on the receptor affinity. Hence, we determined the eutomers and their selectivity indices as well as the eudismic ratio of the pure enantiomers of the lead **VL-0395** and of compounds **8** and **11**.

The binding data reported in Table 2 show that the pharmacologically active enantiomer is the one in which the absolute configuration of the only chiral center of the molecules is R. In fact, compounds **17**, **19** and **21** were about 96-, 199-, and 65-fold more active than their distomers **16**, **18** and **20** respectively.

The highest stereoselectivity (ER = 199) and selectivity versus CCK_1 receptors (SI = 450) was observed for the eutomer of the 5-fluoro derivative (comp. **19**) while the 7-fluoro eutomer (comp. **21**) showed a good receptor selectivity (SI = 360) associated with the highest CCK_1 affinity (IC_{50} = 55 nM) among the N-terminal anthranilic acid derivatives.

Despite the higher affinity of the 7-indole eutomer (comp. **21**), we consider derivative **19** as the best product derived from the N-terminal anthranoyl-phenylalanine optimization step because of its high selectivity, stereoselectivity, its straightforward synthesis and the availability of starting materials.

CONCLUSIONS

Starting from our lead compound, **VL-0395**, an anthranilic acid based CCK_1 receptor antagonist, and following the well established "step by step" lead investigation strategy, we have described the final step of the anthranilic acid N-terminal optimization. A previously reported study on SAfR on the N-terminal substitution enabled us to demonstrate that the 2-indole moiety behaves as a "needle", since any substitution of the indole group with at least 30 different other residues, produces a loss in affinity. In the present study, in order to fine-tune the receptor subsite interaction of the indole moiety of the lead compound, we have synthesized the substituted indole derivatives reported in Table 1. Improvements for both affinity and selectivity toward CCK_1 receptors have been accomplished through introduction of the fluoro substituent at C-5 and C-7 positions of the indole ring and evaluating the best configuration at the aminoacidic chiral center. Moreover, employing the fluoro substituent as a "probe", we have shown that the receptor sub-site tolerance around the indole moiety is extremely limited.

EXPERIMENTAL

Chemical Procedures

All chemicals and solvents used in syntheses were reagent-grade products and were used without additional purification. Reaction progress was monitored by ascen-ding thin-layer chromatography (TLC) using precoated silica gel plates (60F - 254 Merck). Melting points were determined on a Büchi 510 melting point apparatus (Büchi, Flawil, Switzerland) and are uncorrected. Preparative medium-pressure chromatography MPLC was performed on a Büchi 688 apparatus using silica gel (Merck Kieselgel 60, 15 – 40 μm). Optical rotations were determined with a Perkin-Elmer 241 polarimeter in a 1.0 dm tube. Analytical liquid chromatography was performed on a Jasco chromatograph equipped with a Rheodyne Model 7725i 20-μL injector and a Model Jasco PU-1580-CO_2 solvent-delivery system. Different detectors were used, including a Model CD-995 polarimetric and UV detectors (Jasco Europe, Italy) and a Model Sedex-55 evaporative light scattering detection (ELSD) system (S.E.D.E.R.E., France). Column: CSP-TE-SP-100/5 (250 x 4.0, L x I.D. mm), the chiral stationary phase contains native teicoplanin covalently bonded to an aminopropyl-function-alized silica gel, *via* a bifunctional aliphatic isocyanate, according to an efficient "one-pot" synthetic strategy [31]. 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 using $CDCl_3$ or $(CD_3)_2SO$ as solvent. Chemical shifts were reported as parts per million (ppm, δ units) downfield from an internal Me_4Si standard. Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet and b, broad. ^{13}C NMR spectra were determined using either the Attached Proton Test (APT) or standard ^{13}C pulse sequence parameters. Spectral data are consistent with assigned structures. Mass spectra were recorded on an API-1 Perkin-Elmer SCIEX spectrometer by electrospray ionization (ES).

Syntheses

Indole-2-carboxylic acids **1a-8a** were commercially available while all the other ones were prepared as described below:

General Procedure for the Synthesis of 1*H*-Indole-2-Carboxylic Acids 9a and 10a

To a solution of 2.19 g (32.2 mmol) of sodium ethoxide in 10 mL of abs. EtOH was added a mixture of 4.71 g (32.2 mmol) of ethyl oxalate and 5.00 g (32.2 mmol) of 2-fluoro-6-nitrotoluene or 4-fluoro-2-nitrotoluene respectively. The reaction mixture was refluxed for 25 min, then 8 mL of H_2O were added and heating was continued for 1 h [25]. After cooling to 0 °C, the reaction mixture was acidified with conc. HCl keeping the temperature below 10 °C, the solvent was evaporated under vacuum, keeping the temperature below 35 °C. Then diethyl ether was added, the insoluble residue was filtered and the organic phase was extracted 3 times with N NaOH. The combined aqueous phases were washed with diethyl ether and acidified with 6N HCl. The aqueous phase was extracted 3 times with diethyl ether. The combined organic phases were washed with H_2O, brine, dried over anhydrous Na_2SO_4 and rotary evaporated [26]. The residue obtained respectively in 36% and 46% yield, was utilized in the next step without further purification. Reduction was achieved as described by Kermack [27]. 3.34 g (14.7 mmol) of 3-(2-fluoro-6-nitro-phenyl)-2-oxo-propionic acid or 3-(4-fluoro-2-nitro-phenyl)-2-oxo-propionic acid were dissolved in 24 mL of 32% NH_4OH and 35 mL of H_2O. Then a warm solution of 26.45 g (95.1 mmol) of $FeSO_4 \cdot 7H_2O$ was added and the reaction mixture was refluxed for 1 h. The hot reaction mixture was filtered and the solid was washed with hot water. The filtrate was concentrated in vacuo and acidified at 0 °C. The precipitate was collected and purified by trituration with hot EtOH, followed by MPLC (silica gel, 7:3 CH_2Cl_2/AcOEt).

*4-Fluoro-1*H*-Indole-2-Carboxylic Acid (9a)*

Yield: 63%. Molecular formula: $C_9H_6NO_2F$; TLC (AcOEt/MeOH 5:1) – Rf 0.57; mp 210 °C (Lit. [32] 219-220 °C dec). ^{1}H NMR (DMSO-d_6) δ 6.86 (m, 1H, Ar); 7.10 (d, 1H, Ar); 7.24 (m, 2H, Ar); 12.13 (s, 1H, -NH-); ^{13}C NMR (DMSO-d_6) δ 103.27, 104.74, 105.09, 109.74, 109.81, 116. 63, 117.08, 125.54, 125.69, 129.68, 140.11, 140.31, 154.37, 159.27, 163.02.

*6-Fluoro-1*H*-Indole-2-Carboxylic Acid (10a)*

Yield: 53%. Molecular formula: $C_9H_6NO_2F$; TLC (AcOEt/MeOH 5:1) – Rf 0.52; mp 241 °C (Lit. [32] 246 °C

dec). ^{1}H NMR (DMSO-d_6) δ 6.95 (m, 1H, Ar); 7.14 (m, 2H, Ar); 7.67 (m, 1H, Ar); 11.86 (s, 1H, -NH-); ^{13}C NMR (DMSO-d_6) δ 98.30, 98.81, 108.29, 109.72, 110.22, 124.19, 124.40, 124.44, 129.89, 129.96, 137.71, 137.97, 158.68, 163.16, 163.42.

General Procedure for the Synthesis of 1*H*-Indole-2-Carboxylic Acid Ethyl Esters 11b, 12b, 14b and 15b

A solution of 12.3 mmol of the corresponding 2-substituted phenylhydrazine in 8 mL of methanol was gradually added under stirring to a mixture of 1.69 g (14.6 mmol) of ethyl pyruvate and 4.96 g (60.5 mmol) of sodium acetate in 8 mL of H_2O. After stirring at r.t. for 6 h, water was added and the product was collected by filtration and used in the next step without purification.

5.04 g (37.0 mmol) of $ZnCl_2$ were added to 7.14 mmol of the mixture of E and Z phenylhydrazones in 50 mL of glacial acetic acid and the reaction mixture was stirred under reflux until completion (TLC monitoring) [29]. Then H_2O was added and the reaction mixture was extracted with diethyl ether. The organic phase was washed with a saturated solution of sodium bicarbonate, H_2O and brine, dried over anhydrous Na_2SO_4 and rotary evaporated. The residue was purified by flash chromatography using CH_2Cl_2/hexane (from 1:3 to 1:1) as eluent, followed by trituration with petroleum ether 40-60°. For compound **11b** the purification was obtained by flash chromatography eluting with 4% diethyl ether in hexane.

7-Fluoro-1*H-*Indole-2-Carboxylic Acid Ethyl Ester (11b)

MPLC (silica gel, Et_2O/hexane 4%) afforded the titled compound in 30% yield. Molecular formula: $C_{11}H_{10}NO_2F$; TLC (AcOEt/hexane 1:3) – Rf 0.64; mp 139 °C (Lit. [32] 140 °C). ^{1}H NMR ($CDCl_3$) δ 1.43 (t, 3H, -CH_3); 4.43 (q, 2H, -CH_2-O-); 7.05 (m, 2H, Ar); 7.25 (m, 1H, Ar); 7.45 (m, 1H, Ar); 9.12 (s, 1H, -NH-); ^{13}C NMR ($CDCl_3$) δ 14.53, 61.41, 108.99, 109.04, 109.46, 109.77, 118.26, 118.34, 120.90, 121.01, 125.54, 125.81, 128.42, 130.80, 130.90, 147.15, 152.03, 161.56.

5,7-Difluoro-1*H-*Indole-2-Carboxylic Acid Ethyl Ester (12b)

MPLC (silica gel, CH_2Cl_2/hexane 1:1) and trituration with petroleum ether 40-70° afforded the titled compound in 31% yield. Molecular formula: $C_{11}H_9NO_2F_2$; TLC (AcOEt/hexane 1:3) – Rf 0.47; mp 163-164 °C. ^{1}H NMR ($CDCl_3$) δ 1.43 (t, 3H, -CH_3); 4.44 (q, 2H, -CH_2-); 6.87 (t, 1H, Ar); 6.92-7.26 (m, 2H, Ar); 9.33 (s, 1H, -NH-); ^{13}C NMR ($CDCl_3$) δ 14.37, 61.49, 99.93, 100.33, 100.54, 100.94, 102.28, 102.37, 102.74, 102.84, 108.68, 108.74, 108.80, 108.85, 122.27, 122.54, 129.20, 129.32 129.43, 129.61, 146.18, 146.45, 151.12, 151.41, 154.57, 154.76, 159.32, 159.50, 161.29.

7-Methyl-1*H-*Indole-2-Carboxylic Acid Ethyl Ester (14b)

MPLC (silica gel, CH_2Cl_2/hexane 1:1) and trituration with petroleum ether 40-70° afforded the titled compound in 26% yield. Molecular formula: $C_{12}H_{13}NO_2$; TLC (AcOEt/hexane 1:3) – Rf 0.48; mp 128 °C (Lit. [33] 129.5 °C). ^{1}H NMR ($CDCl_3$) δ 1.42 (t, 3H, -CH_2-C<u>H</u>$_3$); 2.51 (s, 3H, -CH_3); 4.41 (q, 2H, -CH_2-); 7.02-7.12 (m, 2H, Ar); 7.24 (s, 1H, Ar); 7.53 (d, 1H, Ar); 9.04 (s, 1H, -NH-); ^{13}C NMR ($CDCl_3$) δ 14.44, 16.73, 61.00, 109.09, 120.10, 120.91, 121.15, 125.43, 126.98, 127.14, 136.64, 162.16.

7-Trifluoromethyl-1*H-*Indole-2-Carboxylic Acid Ethyl Ester (15b)

MPLC (silica gel, CH_2Cl_2/hexane 1:1) and trituration with petroleum ether 40-70° afforded the titled compound in 25% yield. Molecular formula: $C_{12}H_{10}NO_2F_3$; TLC (AcOEt/hexane 1:3) – Rf 0.58; mp 79 °C (Lit. [30] 81.5-82 °C). ^{1}H NMR ($CDCl_3$) δ1.43 (t, 3H, -CH3); 4.44 (q, 2H, -CH2-); 7.19-7.30 (m, 2H, Ar); 7.59 (d, 1H, Ar); 7.88 (d, 1H, Ar); 9.12 (s, 1H, -NH-); ^{13}C NMR ($CDCl_3$) δ14.42, 61.41, 108.64, 113.86, 114.53, 120.07, 121.78, 122.61, 122.70, 122.79, 122.88, 126.63, 127.17, 128.92, 131.97, 132.57, 161.24.

3-Methyl-1*H-*Indole-2-Carboxylic Acid Ethyl Ester (13b)

The above compound was prepared according to the procedure described by Kitano [28]. MPLC (silica gel, CH_2Cl_2/hexane 1:1) and trituration with petroleum ether 40-70° afforded the titled compound in 17% yield. Molecular formula: $C_{12}H_{13}NO_2$; TLC (AcOEt/hexane 1:3) – Rf 0.49; mp 134-135 °C (Lit. [28] 133-134 °C dec). ^{1}H NMR ($CDCl_3$) δ 1.43 (t, 3H, -CH_2-C<u>H</u>$_3$); 2.61 (s, 3H, -CH_3); 4.42 (q, 2H, -CH_2-O-); 7.13 (m, 1H, Ar); 7.25-7.39 (m, 2H, Ar); 7.67 (d, 1H, Ar); 8.74 (s, 1H, -NH-); ^{13}C NMR ($CDCl_3$) δ 10.10, 14.63, 60.80, 111.67, 119.94, 120.22, 120.82, 123.47, 125.60, 128.60, 135.85, 162.70.

General Procedure for the Synthesis of 1*H*-Indole-2-Carboxylic Acids 11a-15a.

A solution of 7.19 mmol of ester (**11b-15b)** in 42 mL of EtOH was added to a solution of 2.02 g (36.0 mmol) of KOH in 42 mL of EtOH, and the reaction mixture was refluxed for 30 min, until completion (TLC monitoring) [29]. EtOH was removed *in vacuo* and H_2O was added. The aqueous phase was washed with diethyl ether, acidified with 2N HCl to pH 3-4 and extracted 3 times with diethyl ether. The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4 and evaporated under vacuum. The residue was triturated with petroleum ether 40-60° at 0 °C and the precipitate collected by filtration. Compound **4a** was purified by MPLC (silica, CH_2Cl_2 to EtOAc/CH_2Cl_2 1:9).

7-Fluoro-1*H-*Indole-2-Carboxylic Acid (11a)

Yield: 91%. Molecular formula: $C_9H_6NO_2F$; TLC (AcOEt/MeOH 5:1) – Rf 0.36; mp 200 °C (Lit. [32] 198 °C dec). ^{1}H NMR (DMSO-d_6) δ 7.07 (m, 2H, Ar); 7.18 (m, 1H, Ar); 7.47 (m, 1H, Ar); 12.26 (s, 1H, -NH-); 13.11 (b, 1H, -OH); ^{13}C NMR (DMSO-d_6) δ 108.90, 108.94, 109.27, 109.59, 118.71, 118.79, 120.85, 120.96, 126.16, 126.44, 130.69, 131.22, 131.33, 147.53, 152.42, 163.08.

5,7-Difluoro-1*H-*Indole-2-Carboxylic Acid (12a)

Yield: 91%. Molecular formula: $C_9H_5NO_2F_2$; TLC (AcOEt/MeOH 5:1) – Rf 0.40; mp 264 °C dec. ^{1}H NMR (DMSO-d_6) δ7.09-7.21 (m, 2H, Ar); 7.30 (d, 1H, Ar); 12.42 (s, 1H, -NH-); 13.24 (b, 1H, -OH); ^{13}C NMR (DMSO-d_6)

δ98.88, 99.29, 99.49, 99.90, 101.86, 101.95, 102.31, 102.40, 108.11, 108.17, 122.26, 122.53, 128.81, 128.95, 129.06, 129.19, 131.32, 145.84, 146.13, 150.80, 151.09, 153.37, 153.57, 158.04, 158.24, 161.95.

3-Methyl-1*H*-Indole-2-Carboxylic Acid (13a)

Yield: 31%. Molecular formula: $C_{10}H_9NO_2$; TLC (AcOEt/hexane 1:1) – Rf 0.35; mp 163-164 °C. ^{1}H NMR (DMSO-d_6) δ 2.53 (s, 3H, -CH_3); 7.04 (t, 1H, Ar); 7.24 (t, 1H, Ar); 7.39 (d, 1H, Ar); 7.63 (d, 1H, Ar); 11.37 (s, 1H, -NH-); 12.88 (b, 1H, -OH); ^{13}C NMR (DMSO-d_6) δ 9.99, 112.39, 117.89, 119.24, 120.34, 124.19, 124.69, 127.93, 136.19, 163.66

7-Methyl-1*H*-Indole-2-Carboxylic Acid (14a)

Yield: 86%. Molecular formula: $C_{10}H_9NO_2$; TLC (AcOEt/MeOH 5:1) – Rf 0.53; mp 174 °C (Lit. [33] 173-173.5 °C). ^{1}H NMR (DMSO-d_6) δ 2.51 (s, 3H, -CH_3); 6.93-7.04 (m, 2H, Ar); 7.10 (s, 1H, Ar); 7.46 (d, 1H, Ar); 11.61 (s, 1H, -NH-); 12.92 (b, 1H, -OH); ^{13}C NMR (DMSO-d_6) δ 17.12, 108.03, 119.26, 120.07, 121.98, 124.54, 126.54, 128.35, 136.87, 162.71.

7-Trifluoromethyl-1*H*-Indole-2-Carboxylic Acid (15a)

Yield: 22%. Molecular formula: $C_{10}H_6NO_2F_3$; TLC (AcOEt/MeOH 5:1) – Rf 0.24; mp 210 °C dec (Lit. [29] 195 °C). ^{1}H NMR (DMSO-d_6) δ 7.22-7.29 (m, 2H, Ar); 7.63 (d, 1H, Ar); 7.99 (d, 1H, Ar); 11.86 (s, 1H, -NH-); 13.25 (b, 1H, - OH); ^{13}C NMR (DMSO-d_6) δ 108.48, 112.99, 113.64, 119.33, 121.21, 121.81, 121.91, 126.61, 126.75, 128.65, 130.71, 131.70, 162.00.

General Procedure for the Syntheses of Anthranoyl-Phenylalanine Ethyl Esters (22-24)

A suspension of the corresponding (DL-, L- or D-) phenylalanine ethyl ester hydrochloride (5.00 g, 21.8 mmol) in 150 mL of ethyl acetate was treated with triethylamine (3.07 mL, 21.8 mmol) followed by isatoic anhydride (3.55 g, 21.8 mmol). The resulting mixture was refluxed under stirring for 2 h, cooled to room temperature and filtered. The organic phase was thoroughly washed with N NaOH (2 x 50 mL), water (2 x 50 mL) and brine, dried over anhydrous sodium sulphate and concentrated *in vacuo*.

2 (R,S)-(2-Amino-Benzoylamino)-3-Phenyl-Propionic Acid Ethyl Ester (22)

Trituration with petroleum ether 40-70° afforded the analytically pure title compound in 80% yield. Molecular formula: $C_{18}H_{20}N_2O_3$; TLC (AcOEt/hexane 1:1) – Rf 0.69; mp 84-85 °C; ^{1}H NMR ($CDCl_3$) δ 1.24 (t, 3H, -CH_3); 3.21 (m, 2H, -C<u>H</u>$_2$-CH<); 4.18 (q, 2H, -CH_2-O-); 4.97 (m, 1H, >CH-); 5.45 (s, 2H, -NH_2); 6.52 (d, 1H, -NH-); 6.61-7.28 (m, 9H, Ar).^{13}C NMR ($CDCl_3$) δ 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.

2(S)-(2-Amino-Benzoylamino)-3-Phenyl-Propionic Acid Ethyl Ester (23)

MPLC (silica gel; elution: from CH_2Cl_2 to AcOEt/ CH_2Cl_2 1:4) afforded an oil which crystallize with cold hexane to give the titled compound in 62% yield. Mole-cular formula: $C_{18}H_{20}N_2O_3$; TLC (AcOEt/hexane 1:1) – Rf 0.69; mp 89-90 °C (Lit. [34,35] 88-90 °C; 82-84 °C); $[a]_D^{25}$ +83.1 (*c* 2.1, CH_2Cl_2) [Lit. [34] +76.8 (*c* 2, CH_2Cl_2)]; ^{1}H NMR ($CDCl_3$) δ 1.27 (t, 3H, -CH_3); 3.23 (m, 2H, -C<u>H</u>$_2$-CH<); 4.20 (q, 2H, -CH_2-O-); 5.00 (m, 1H, -CH<); 5.47 (b, 2H, -NH_2); 6.51 (d, 1H, -NH-); 6.64 (m, 2H, Ar); 7.14-7.28 (m, 7H, Ar); ^{13}C NMR ($CDCl_3$) δ 14.30, 38.13, 53.41, 61.69, 115.42, 116.71, 117.30, 127.16, 127.43, 128.61, 129.44, 132.60, 136.03, 148.84, 168.61, 171.69

2(R)-(2-Amino-Benzoylamino)-3-Phenyl-Propionic Acid Ethyl Ester (24)

MPLC (silica gel; elution: from CH_2Cl_2 to AcOEt/ CH_2Cl_2 1:4) afforded an oil which crystallize with cold hexane to give the titled compound in 49% yield. Molecular formula: $C_{18}H_{20}N_2O_3$; TLC (AcOEt/hexane 1: 1) – Rf 0.69; mp 82-83 °C; $[a]_D^{25}$ -84.1 (*c* 2.1 CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 1.27 (t, 3H, -CH_3); 3.23 (m, 2H, -C<u>H</u>$_2$-CH<); 4.20 (q, 2H, -CH_2-O-); 5.00 (m, 1H, -CH<); 5.47 (b, 2H, -NH_2); 6.51 (d, 1H, -NH-); 6.65 (m, 2H, Ar); 7.14-7.35 (m, 7H, Ar); ^{13}C NMR ($CDCl_3$) δ 14.30, 38.14, 53.41, 61.70, 115.42, 116.71, 117.30, 127.17, 127.43, 128.61, 129.44, 132.61, 136.02, 148.84, 168.61, 171.69

General Coupling Procedure of Anthranoyl-Phenylalanine Ethyl Ester (1c-21c)

To a suspension of 5.0 mmol of the corresponding indole-2-carboxylic acid in 20 mL of acetyl chloride cooled in an ice-bath were added portionwise, over a period of 0.5 h, 5.0 mmol (1.04 g) of PCl_5. After the mixture turned into a clear solution, stirring was continued at room temperature for 2 hours. The solution was concentrated under reduced pressure and the residue, taken up in 5 mL of dry CH_2Cl_2, was added dropwise at 0 °C to a solution of 4.0 mmol of the corresponding anthranoyl-phenylalanine ethyl ester (**22**, **23** or **24**) in 4 mL of pyridine. After the addition was completed, the reaction mixture was stirred at room temperature overnight. Then 50 mL of CH_2Cl_2 were added and the organic layer was washed twice with 40 mL of N HCl, H_2O, 0.1N NaOH and brine. After drying over Na_2SO_4, the organic phase was rotary evaporated and the residue was purified as described to yield the titled compounds.

2(R,S)-{2-[(5-Methyl-1*H*-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid Ethyl Ester (1c)

MPLC (silica gel; CH_2Cl_2/AcOEt 4:1) afforded the titled compound in 78% yield. Molecular formula: $C_{28}H_{27}N_3O_4$; TLC (AcOEt/hexane 1:1) – Rf 0.72; mp 230 °C; ^{1}H NMR (DMSO-d_6) δ 1.14 (t, 3H, -CH_2-C<u>H</u>$_3$); 2.38 (s, 3H, -CH_3); 3.19 (m, 2H, -C<u>H</u>$_2$-CH<); 4.12 (q, 2H, -CH_2-O-); 4.75 (m, 1H, -CH<); 6.86 (s, 1H, Ar); 7.06-7.38 (m, 8H, Ar); 7.46 (s, 1H, Ar); 7.59 (t, 1H, Ar); 7.77 (d, 1H, Ar); 8.61 (d, 1H, Ar); 9.29 (d, 1H, -N<u>H</u>-CH<); 11.81 (s, 1H, -NH-); 12.07 (s, 1H, -NH-); ^{13}C NMR (DMSO-d_6) δ 14.81, 21.98, 36.82, 55.13, 61.54, 102.79, 112.95, 119.99, 120.61, 121.63, 123.24, 126. 73, 127.25, 127.86, 128.93, 129.16, 129.55, 129.80, 132.13, 133.36, 136.26, 138.16, 139.84, 159.82, 169.57, 171.89.

2(R,S)-{2-[(5-Methoxy-1*H*-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid Ethyl Ester (2c)

MPLC (silica gel; CH_2Cl_2/AcOEt 4:1) afforded the titled compound in 82% yield. Molecular formula: $C_{28}H_{27}N_3O_5$; TLC (AcOEt/hexane 1:1) –Rf 0.59; mp 204-205 °C; ^{1}H NMR (DMSO-d_6) δ 1.15 (t, 3H, -CH_2-C<u>H</u>$_3$); 3.20 (m, 2H, -C<u>H</u>$_2$-CH<); 3.78 (s, 3H, -O-CH_3); 4.13 (q, 2H, -CH_2-O-); 4.75 (m, 1H, -CH<); 6.89 (m, 2H, Ar); 7.17-7.38 (m, 8H, Ar); 7.59 (t, 1H, Ar); 7.79 (d, 1H, Ar); 8.61 (d, 1H, Ar); 9.29 (d, 1H, -N<u>H</u>-CH<); 11.79 (s, 1H, -NH-); 12.12 (s, 1H, -NH-); ^{13}C NMR (DMSO-d_6) δ 14.81, 36.81, 55.13, 55.97, 61.57, 102.66, 103.02, 114.06, 116.20, 119.83, 120.56, 123.22, 127.26, 127.96, 128.95, 129.15, 129.79, 132.41, 133.13, 133.39, 138.17, 139.90, 154.67, 159.72, 169.57, 171.89.

2(R,S)-{2-[(5,6-Dimethoxy-1*H*-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid Ethyl Ester (3c)

MPLC (silica gel; CH_2Cl_2/AcOEt 4:1) afforded the titled compound in 72% yield. Molecular formula: $C_{29}H_{29}N_3O_6$; TLC (AcOEt/hexane 1:1) – Rf 0.33; mp 201-202 °C; ^{1}H NMR (DMSO-d_6) δ 1.15 (t, 3H, -CH_2-C<u>H</u>$_3$); 3.19 (m, 2H, -C<u>H</u>$_2$-C_6H_5); 3.79 (s, 6H, -O-CH_3); 4.13 (q, 2H, -CH_2-O-); 4.74 (m, 1H, -CH<); 6.91 (m, 2H, Ar); 7.17-7.36 (m, 7H, Ar); 7.58 (t, 1H, Ar); 7.78 (d, 1H, Ar); 8.61 (d, 1H, Ar); 9.27 (d, 1H, -N<u>H</u>-CH<); 11.64 (s, 1H, -NH-); 12.02 (s, 1H, -NH-); ^{13}C NMR (DMSO-d_6) δ 14.81, 36.81, 55.13, 56.16, 56.36, 61.57, 95.12, 103.18, 103.65, 119.63, 120.51, 122.93, 127.27, 128.96, 129.15, 129.79, 130.34, 132.99, 133.37, 138.18, 140.18, 146.31, 149.61, 159.73, 169.63, 171.92.

2 (R,S)- {2 -[(5-Benzyloxy-1*H*-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid Ethyl Ester (4c)

MPLC (silica gel; CH_2Cl_2/AcOEt 4:1) afforded the titled compound in 77% yield. Molecular formula: $C_{34}H_{31}N_3O_5$; TLC (AcOEt/hexane 1:1) – Rf 0.45; mp 239-240 °C; ^{1}H NMR (DMSO-d_6) δ 1.14 (t, 3H, -CH_3); 3.19 (m, 2H, -C<u>H</u>$_2$-CH<); 4.12 (q, 2H, -CH_2-O-); 4.74 (m, 1H, -CH<); 5.12 (s, 2H, -O-C<u>H</u>$_2$-C_6H_5); 6.86 (s, 1H, Ar); 6.96 (m, 1H, Ar); 7.00-7.50 (m, 13H, Ar); 7.59 (t, 1H, Ar); 7.78 (d, 1H, Ar); 8.61 (d, 1H, Ar); 9.29 (d, 1H, -N<u>H</u>-CH<); 11.80 (s, 1H, -NH-); 12.09 (s, 1H, -NH-); ^{13}C NMR (DMSO-d_6) δ 14.80, 36.82, 55.15, 61.57, 70.28, 103.03, 104.19, 114.13, 116.72, 119.86, 120.56, 123.23, 127.26, 127.90, 128.38, 128.94, 129.08, 129.79, 132.51, 133.26, 133.38, 138.17, 139.90, 153.65, 159.70, 169.58, 171.90.

2(R,S)-{2-[(5-Chloro-1*H*-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid Ethyl Ester (5c)

MPLC (silica gel; CH_2Cl_2/AcOEt 4:1) afforded the titled compound in 89% yield. Molecular formula: $C_{27}H_{24}ClN_3O_5$; TLC (AcOEt/hexane 1:1) – Rf 0.63; mp 165 °C; ^{1}H NMR (DMSO-d_6) δ 1.14 (t, 3H, -CH_3); 3.19 (m, 2H, -C<u>H</u>$_2$-CH<); 4.12 (q, 2H, -CH_2-O-); 4.76 (m, 1H, -CH<); 6.95 (s, 1H, Ar); 7.12-7.35 (m, 7H, Ar); 7.48 (d, 1H, Ar); 7.60 (t, 1H, Ar); 7.80 (m, 2H, Ar); 8.59 (d, 1H, Ar); 9.30 (d, 1H, -N<u>H</u>-CH<); 12.15 (s, 2H, -NH-); ^{13}C NMR (DMSO-d_6) δ 14.80, 36.85, 55.11, 61.58, 102.83, 114.84, 120.10, 120.80, 121.59, 123.51, 124.95, 125.39, 127.24, 128.66, 128.93, 129.19, 129.80, 133.40, 133.62, 136.17, 138.14, 139.64, 159.39, 169.51, 171.87.

2(R,S)-{2-[(5-Nitro-1*H*-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid Ethyl Ester (6c)

MPLC (silica gel; elution: from CH_2Cl_2 to AcOEt/CH_2Cl_2 1:4) afforded the titled compound in 64% yield. Molecular formula: $C_{27}H_{24}N_4O_6$; TLC (AcOEt/hexane 1: 1) – Rf 0.54; mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 1.14 (t, 3H, -CH_3); 3.19 (m, 2H, -C<u>H</u>$_2$-CH<); 4.12 (q, 2H, -CH_2-O-); 4.77 (m, 1H, -CH<); 7.11-7.35 (m, 7H, Ar); 7.62 (m, 2H, Ar); 7.82 (d, 1H, Ar); 8.13 (m, 1H, Ar); 8.57 (d, 1H, Ar); 8.81 (m, 1H, Ar); 9.31 (d, 1H, -N<u>H</u>-CH<); 12.24 (s, 1H, -NH-); 12.64 (s, 1H, -NH-); ^{13}C NMR (DMSO-d_6) δ 14.78, 36.90, 55.07, 61.60, 105.70, 113.76, 119.70, 120.23, 120.34, 121.03, 123.81, 126.92, 127.23, 128.93, 129.19, 129.80, 133.41, 135.64, 138.12, 139.40, 140.51, 142.18, 158.95, 169.43, 171.84.

2(R,S)-{2-[(1-Methyl-5-Nitro-1*H*-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid Ethyl Ester (7c)

MPLC (silica gel; elution: from CH_2Cl_2 to AcOEt/CH_2Cl_2 1:4) afforded the titled compound in 80% yield. Molecular formula: $C_{28}H_{26}N_4O_6$; TLC (AcOEt/hexane 1: 1) – Rf 0.68; mp 229 °C; ^{1}H NMR (DMSO-d_6) δ 1.13 (t, 3H, -CH_2-C<u>H</u>$_3$); 3.18 (m, 2H, -C<u>H</u>$_2$-CH<); 4.09 (s, 3H, >N-C<u>H</u>$_3$); 4.12 (q, 2H, -CH_2-O-); 4.75 (m, 1H, -CH<); 7.11-7.35 (m, 7H, Ar); 7.60 (t, 1H, Ar); 7.81 (m, 2H, Ar); 8.17 (m, 1H, Ar); 8.52 (d, 1H, Ar); 8.76 (m, 1H, Ar); 9.30 (d, 1H, -N<u>H</u>-CH<); 12.10 (s, 1H, -NH-); ^{13}C NMR (DMSO-d_6) δ 14.78, 33.03, 36.89, 55.05, 61.57, 107.60, 112.33, 119.59, 119.99, 120.66, 121.04, 123.90, 125.30, 127.22, 128.92, 129.16, 129.80, 133.29, 136.10, 138.14, 139.33, 142.03, 142.32, 159.61, 169.35, 171.85.

2(R,S)-{2-[(5-Fluoro-1*H*-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid Ethyl Ester (8c)

MPLC (silica gel; elution: from CH_2Cl_2 to AcOEt/CH_2Cl_2 1:4) afforded the titled compound in 84% yield. Molecular formula: $C_{27}H_{24}FN_3O_5$; TLC (AcOEt/hexane 1: 1) – Rf 0.54; mp 248 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.14 (t, 3H, -CH_3); 3.20 (m, 2H, -C<u>H</u>$_2$-CH<); 4.12 (q, 2H, -CH_2-O-); 4.76 (m, 1H, -CH<); 6.95 (s, 1H, Ar); 7.06-7.35 (m, 7H, Ar); 7.44-7.64 (m, 3H, Ar); 7.80 (d, 1H, Ar); 8.60 (d, 1H, Ar); 9.29 (d, 1H, -N<u>H</u>-CH<); 12.04 (s, 1H, -NH-); 12.15 (s, 1H, -NH-); ^{13}C NMR (DMSO-d_6) δ 14.00, 36.05, 54.32, 60.78, 102.43, 102.54, 105.63, 106.10, 112.59, 113.13, 113.57, 113.77, 119.22, 119.91, 122.64, 126.42, 126.81, 127.03, 128.12, 128.36, 128.98, 132.60, 133.02, 133.73, 137.34, 138.89, 154.86, 158.64, 159.48, 168.71, 171.06.

2(R,S)-{2-[(4-Fluoro-1*H*-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid Ethyl Ester (9c)

MPLC (silica gel; elution: from CH_2Cl_2 to AcOEt/CH_2Cl_2 1:4) afforded the titled compound in 86% yield. Molecular formula: $C_{27}H_{24}FN_3O_5$; TLC (AcOEt/hexane 1: 1) – Rf 0.57; mp 225 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.14 (t, 3H, -CH_3); 3.18 (m, 2H, -C<u>H</u>$_2$-CH<); 4.12 (q, 2H, -CH_2-O-); 4.75 (m, 1H, -CH<); 6.89 (m, 1H, Ar); 6.96 (s, 1H, Ar); 7.12-7.36 (m, 8H, Ar); 7.61 (t, 1H, Ar); 7.77 (d, 1H, Ar); 8.59 (d, 1H, Ar); 9.32 (d, 1H, -N<u>H</u>-CH<); 12.15 (s, 1H, -NH-); 12.29 (s, 1H, -NH-); ^{13}C NMR (DMSO-d_6) δ 14.77, 36.81, 55.25, 61.51, 98.73, 105.00, 105.37, 109.82, 109.88, 116.66,

117.09, 120.27, 120.70, 123.54, 125.35, 125.51, 127.24, 128.91, 129.20, 129.82, 132.75, 133.39, 138.13, 139.57, 140.03, 140.23, 154.30, 159.23, 169.60, 171.93.

2(R,S)-{2-[(6-Fluoro-1H-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid Ethyl Ester (10c)

MPLC (silica gel; elution: from CH_2Cl_2 to AcOEt/ CH_2Cl_2 1:4) afforded the titled compound in 60% yield. Molecular formula: $C_{27}H_{24}FN_3O_5$; TLC (AcOEt/hexane 1: 1) – Rf 0.55; mp 238-239 °C; ^{1}H NMR (DMSO-d_6) δ 1.14 (t, 3H, -CH_3); 3.19 (m, 2H, -C<u>H</u>$_2$-CH<); 4.12 (q, 2H, -CH_2-O-); 4.76 (m, 1H, -CH<); 6.98 (m, 2H, Ar); 7.12-7.35 (m, 7H, Ar); 7.60 (t, 1H, Ar); 7.76 (m, 2H, Ar); 8.59 (d, 1H, Ar); 9.29 (d, 1H, -N<u>H</u>-CH<); 12.01 (s, 1H, -NH-); 12.10 (s, 1H, -NH-); ^{13}C NMR (DMSO-d_6) δ 14.80, 36.84, 55.11, 61.56, 98.40, 98.90, 103.56, 109.95, 110.44, 120.03, 120.68, 123.37, 124.07, 124.28, 124.51, 127.24, 128.93, 129.16, 129.79, 132.95, 133.02, 133.39, 137.63, 137.89, 138.15, 139.74, 158.55, 159.46, 163.29, 169.54, 171.88.

2(R,S)-{2-[(7-Fluoro-1H-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid Ethyl Ester (11c)

MPLC (silica gel; elution: from CH_2Cl_2 to AcOEt/ CH_2Cl_2 1:4) afforded the titled compound in 32% yield. Molecular formula: $C_{27}H_{24}FN_3O_5$; TLC (AcOEt/hexane 1: 1) – Rf 0.49; mp 176-177 °C; ^{1}H NMR (DMSO-d_6) δ 1.14 (t, 3H, -CH_3); 3.18 (m, 2H, -C<u>H</u>$_2$-CH<); 4.12 (q, 2H, -CH_2-O-); 4.75 (m, 1H, -CH<); 7.04-7.35 (m, 9H, Ar); 7.52-7.64 (m, 2H, Ar); 7.78 (d, 1H, Ar); 8.58 (d, 1H, Ar); 9.29 (d, 1H, -N<u>H</u>-CH<); 12.10 (s, 1H, -NH-); 12.43 (s, 1H, -NH-); ^{13}C NMR (DMSO-d_6) δ 14.80, 36.86, 55.12, 61.55, 104.35, 109.21, 109.52, 118.57, 120.27, 120.79, 121.17, 121.29, 123.52, 126.04, 126.31, 127.24, 128.92, 129.17, 129.80, 131.27, 131.38, 133.37, 133.75, 138.14, 139.60, 147.52, 152.41, 159.30, 169.49, 171.88.

2(R,S)-{2-[(5,7-Difluoro-1H-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid Ethyl Ester (12c)

MPLC (silica gel; elution: from CH_2Cl_2 to AcOEt/ CH_2Cl_2 1:4) afforded the titled compound in 74% yield. Molecular formula: $C_{27}H_{23}F_2N_3O_5$; TLC (AcOEt/ hexane 1: 1) – Rf 0.67; mp 219-220 °C; ^{1}H NMR (DMSO-d_6) δ1.13 (t, 3H, -CH_3); 3.18 (m, 2H, -C<u>H</u>$_2$-CH<); 4.11 (q, 2H, -C<u>H</u>$_2$-CH_3); 4.75 (m, 1H, -CH<); 7.02 (s, 1H, Ar); 7.10-7.35 (m, 7H, Ar); 7.41 (d, 1H, Ar); 7.61 (t, 1H, Ar); 7.79 (d, 1H, Ar); 8.57 (d, 1H, Ar); 9.30 (d, 1H, -N<u>H</u>-CH<); 12.13 (s, 1H, -NH-); 12.57 (s, 1H, -NH-); ^{13}C NMR (DMSO-d_6) δ13.96, 36.03, 54.26, 60.74, 98.79, 99.19, 99.81, 101.92, 102.37, 103.59, 119.45, 120.03, 122.14, 122.40, 122.82, 126.41, 128.10, 128.35, 128.97, 129.35, 132.57, 134.34, 137.31, 138.69, 146.13, 151.09, 153.57, 153.76, 158.17, 168.62, 171.02.

2(R,S)-{2-[(3-Methyl-1H-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid Ethyl Ester (13c)

MPLC (silica gel; elution: from CH_2Cl_2 to AcOEt/ CH_2Cl_2 1:5) afforded the titled compound in 68% yield. Molecular formula: $C_{28}H_{27}N_3O_4$; TLC (AcOEt/hexane 1: 1) – Rf 0.64; mp 164-165 °C; ^{1}H NMR (DMSO-d_6) δ 1.08 (t, 3H, -CH_2-C<u>H</u>$_3$); 2.50 (s, 3H, -CH_3); 3.14 (m, 2H, -C<u>H</u>$_2$-CH<); 4.06 (q, 2H, -CH_2-O-); 4.67 (m, 1H, -CH<); 7.04-7.13 (m, 2H, Ar); 7.19-7.32 (m, 6H, Ar); 7.42 (d, 1H, Ar); 7.58 (t, 1H, Ar); 7.67 (m, 2H, Ar); 8.47 (d, 1H, Ar); 9.23 (d, 1H, -N<u>H</u>-CH<); 11.16 (s, 1H, -NH-); 11.48 (s, 1H, -NH-); ^{13}C NMR (DMSO-d_6) δ 9.84, 14.12, 36.27, 54.43, 60.90, 112.29, 113.07, 119.42, 120.19, 121.20, 121.41, 122.98, 124.38, 126.58, 128.00, 128.24, 128.30, 128.41, 129.18, 132.22, 136.03, 137.55, 138.52, 160.27, 168.54, 171.31.

2(R,S)-{2-[(7-Methyl-1H-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid Ethyl Ester (14c)

MPLC (silica gel; CH_2Cl_2/AcOEt 4:1) afforded the titled compound in 70% yield. Molecular formula: $C_{28}H_{27}N_3O_4$; TLC (AcOEt/hexane 1:1) – Rf 0.64; mp 124 °C; ^{1}H NMR (DMSO-d_6) δ1.15 (t, 3H, -CH_2-C<u>H</u>$_3$); 2.54 (s, 3H, -CH_3); 3.22 (m, 2H, -C<u>H</u>$_2$-CH<); 4.13 (q, 2H, -C<u>H</u>$_2$-CH_3); 4.76 (m, 1H, -CH<); 6.97-7.36 (t, 9H, Ar); 7.51-7.64 (m, 2H, Ar); 7.78 (d, 1H, Ar); 8.63 (d, 1H, Ar); 9.29 (d, 1H, -N<u>H</u>-CH<); 11.77 (s, 1H, -NH-); 12.09 (s, 1H, -NH-); ^{13}C NMR (DMSO-d_6) δ13.87, 17.00, 35.96, 54.19, 60.59, 103.30, 118.97, 119.26, 119.67, 120.27, 121.93, 122.31, 124.36, 126.30, 126.55, 127.99, 128.24, 128.85, 131.48, 132.38, 136.65, 137.23, 138.94, 158.95, 168.63, 170.94.

3-Phenyl-2(R,S)-{2-[(7-Trifluoromethyl-1H-Indole-2-Carbonyl)-Amino]-Benzoylamino}-Propionic Acid Ethyl Ester (15c)

MPLC (silica gel; CH_2Cl_2/AcOEt 4:1) afforded the titled compound in 58% yield. Molecular formula: $C_{28}H_{24}F_3N_3O_4$; TLC (AcOEt/hexane 1:1) – Rf 0.70; mp 181 °C; ^{1}H NMR (DMSO-d_6) δ1.12 (t, 3H, -CH_3); 3.17 (m, 2H, -C<u>H</u>$_2$-CH<); 4.10 (q, 2H, -C<u>H</u>$_2$-CH_3); 4.75 (m, 1H, -CH<); 7.13-7.30 (m, 8H, Ar); 7.61 (m, 2H, Ar); 7.78 (d, 1H, Ar); 8.05 (d, 1H, Ar); 8.54 (d, 1H, Ar); 9.27 (d, 1H, -N<u>H</u>-CH<); 11.91 (s, 1H, -NH-); 12.04 (s, 1H, -NH-); ^{13}C NMR (DMSO-d_6) δ13.91, 36.04, 54.23, 60.68, 104.11, 112.96, 113.62, 119.64, 119.97, 120.19, 121.26, 121.73, 122.88, 126.36, 126.62, 128.06, 128.32, 128.69, 128.93, 131.56, 132.44, 133.82, 137.28, 138.53, 158.28, 168.53, 170.99.

2(S)-{2-[(1H-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid Ethyl Ester (16c)

MPLC (silica gel; elution: from CH_2Cl_2 to AcOEt/ CH_2Cl_2 1:4) afforded the titled compound in 80% yield. Molecular formula: $C_{27}H_{25}N_3O_4$; TLC (AcOEt/hexane 1: 1) – Rf 0.69; mp 209-210 °C; $[a]_D^{25}$ +44.6 (*c* 0.54, CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 1.14 (t, 3H, -CH_3); 3.19 (m, 2H, -C<u>H</u>$_2$-CH<); 4.12 (q, 2H, -CH_2-O-); 4.76 (m, 1H, -CH<); 6.95 (s, 1H, Ar); 7.06-7.36 (m, 8H, Ar); 7.47 (d, 1H, Ar); 7.60 (t, 1H, Ar); 7.71 (d, 1H, Ar); 7.78 (d, 1H, Ar); 8.61 (d, 1H, Ar); 9.29 (d, 1H, -N<u>H</u>-CH<); 11.92 (s, 1H, -NH-); 12.12 (s, 1H, -NH-); ^{13}C NMR (DMSO-d_6) δ 14.80, 36.84, 55.13, 61.55, 103.33, 113.22, 120.03, 120.64, 120.95, 122.48, 123.30, 124.82, 127.25, 127.63, 128.93, 129.16, 129.80, 132.19, 133.38, 137.81, 138.16, 139.81, 159.77, 169.57, 171.89.

2(R)-{2-[(1H-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid Ethyl Ester (17c)

MPLC (silica gel; elution: from CH_2Cl_2 to AcOEt/ CH_2Cl_2 1:4) afforded the titled compound in 81% yield.

Molecular formula: $C_{27}H_{25}N_3O_4$; TLC (AcOEt/hexane 1: 1) – Rf 0.69; mp 209-210 °C; $[a]_D^{25}$ -44.8 (*c* 0.54, CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 1.14 (t, 3H, -CH_3); 3.19 (m, 2H, -C<u>H</u>$_2$-CH<); 4.12 (q, 2H, -CH_2-O-); 4.76 (m, 1H, -CH<); 6.96 (s, 1H, Ar); 7.06-7.36 (m, 8H, Ar); 7.47 (d, 1H, Ar); 7.60 (t, 1H, Ar); 7.71 (d, 1H, Ar); 7.78 (d, 1H, Ar); 8.62 (d, 1H, Ar); 9.29 (d, 1H, -N<u>H</u>-CH<); 11.92 (s, 1H, -NH-); 12.12 (s, 1H, -NH-); ^{13}C NMR (DMSO-d_6) δ 14.80, 36.82, 55.14, 61.56, 103.31, 113.21, 119.98, 120.62, 120.94, 122.48, 123.31, 124.82, 127.25, 127.61, 128.93, 129.16, 129.80, 132.17, 133.38, 137.80, 138.16, 139.80, 159.76, 169.57, 171.90.

*2 (S) - {2-[(5-Fluoro-1*H*-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid Ethyl Ester (18c)*

MPLC (silica gel; elution: from CH_2Cl_2 to AcOEt/CH_2Cl_2 1:10) afforded the titled compound in 65% yield. Molecular formula: $C_{27}H_{24}FN_3O_4$; TLC (AcOEt/hexane 1:1) – Rf 0.54; mp 250-251 °C; $[a]_D^{25}$ -19.5 (*c* 0.63, DMF); ^{1}H NMR (DMSO-d_6) δ 1.14 (t, 3H, -CH_3); 3.19 (m, 2H, -C<u>H</u>$_2$-CH<); 4.12 (q, 2H, -CH_2-O-); 4.78 (m, 1H, -CH<); 6.95 (s, 1H, Ar); 7.12-7.64 (m, 10H, Ar); 7.80 (d, 1H, Ar); 8.61 (d, 1H, Ar); 9.30 (d, 1H, -N<u>H</u>-CH<); 12.04 (s, 1H, -NH-); 12.15 (s, 1H, -NH-); ^{13}C NMR (DMSO-d_6) δ 13.95, 36.02, 54.26, 60.73, 102.39, 102.49, 105.61, 106.07, 112.56, 113.09, 113.54, 113.74, 119.23, 119.88, 122.61, 126.40, 126.78, 126.99, 128.09, 128.33, 128.95, 132.56, 132.99, 133.70, 137.31, 138.85, 154.82, 158.61, 159.46, 168.68, 171.02.

*2(R)-{2-[(5-Fluoro-1*H*-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid Ethyl Ester (19c)*

Trituration with hot MeOH then with chloroform afforded the analytically pure titled compound in 56% yield. Molecular formula: $C_{27}H_{24}FN_3O_4$; TLC (AcOEt/ hexane 1:1) – Rf 0.54; mp 250-251 °C; $[a]_D^{25}$ +19.0 (*c* 0.62, DMF); ^{1}H NMR (DMSO-d_6) δ 1.14 (t, 3H, -CH_3); 3.19 (m, 2H, -C<u>H</u>$_2$-CH<); 4.12 (q, 2H, -CH_2-O-); 4.77 (m, 1H, -CH<); 6.95 (s, 1H, Ar); 7.06-7.64 (m, 10H, Ar); 7.79 (d, 1H, Ar); 8.60 (d, 1H, Ar); 9.30 (d, 1H, -N<u>H</u>-CH<); 12.04 (s, 1H, -NH-); 12.15 (s, 1H, -NH-); ^{13}C NMR (DMSO-d_6) δ 13.96, 36.01, 54.26, 60.73, 102.38, 102.49, 105.62, 106.07, 112.56, 113.09, 113.54, 113.74, 119.21, 119.88, 122.61, 126.40, 126.78, 126.99, 128.09, 128.33, 128.96, 132.56, 132.99, 133.70, 137.31, 138.86, 154.82, 158.61, 159.46, 168.68, 171.03.

*2(S)-{2-[(7-Fluoro-1*H*-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid Ethyl Ester (20c)*

MPLC (silica gel; elution: from CH_2Cl_2 to AcOEt/CH_2Cl_2 1:4) afforded the titled compound in 34% yield. Molecular formula: $C_{27}H_{24}FN_3O_5$; TLC (AcOEt/hexane 1: 1) – Rf 0.65; mp 179-180 °C; $[a]_D^{25}$ -19.3 (*c* 0.61, DMF); ^{1}H NMR (DMSO-d_6) δ 1.13 (t, 3H, -CH_3); 3.18 (m, 2H, -C<u>H</u>$_2$-CH<); 4.11 (q, 2H, -C<u>H</u>$_2$-CH_3); 4.75 (m, 1H, -CH<); 7.04-7.34 (m, 9H, Ar); 7.52-7.64 (m, 2H, Ar); 7.78 (d, 1H, Ar); 8.57 (d, 1H, Ar); 9.28 (d, 1H, -N<u>H</u>-CH<); 12.08 (s, 1H, -NH-); 12.41 (s, 1H, -NH-); ^{13}C NMR (DMSO-d_6) δ13.94, 36.03, 54.27, 60.71, 103.53, 108.37, 108.68, 117.79, 119.52, 119.98, 120.34, 120.45, 122.69, 125.20, 125.47, 126.39, 128.08, 128.31, 128.95, 130.45, 130.56, 132.50, 132.92, 137.30, 138.74, 146.69, 151.58, 158.47, 168.66, 171.02.

*2(R)-{2-[(7-Fluoro-1*H*-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid Ethyl Ester (21c)*

MPLC (silica gel; elution: from CH_2Cl_2 to AcOEt/CH_2Cl_2 1:4) afforded the titled compound in 82% yield. Molecular formula: $C_{27}H_{24}FN_3O_5$; TLC (AcOEt/hexane 1: 1) – Rf 0.65; mp 180-181 °C; $[a]_D^{25}$ +20.7 (*c* 0.61, DMF); ^{1}H NMR (DMSO-d_6) δ1.14 (t, 3H, -CH_3); 3.18 (m, 2H, -C<u>H</u>$_2$-CH<); 4.12 (q, 2H, -C<u>H</u>$_2$-CH_3); 4.76 (m, 1H, -CH<); 7.04-7.35 (m, 9H, Ar); 7.50-7.64 (m, 2H, Ar); 7.79 (d, 1H, Ar); 8.58 (d, 1H, Ar); 9.28 (d, 1H, -N<u>H</u>-CH<); 12.09 (s, 1H, -NH-); 12.42 (s, 1H, -NH-); ^{13}C NMR (DMSO-d_6) δ13.91, 36.02, 54.24, 60.68, 103.55, 108.35, 108.66, 117.70, 117.77, 119.51, 119.96, 120.31, 120.43, 122.66, 125.18, 125.45, 126.36, 128.06, 128.29, 128.92, 130.43, 130.54, 132.46, 132.92, 137.28, 138.74, 146.67, 151.56, 158.44, 168.63, 170.99.

General Procedure for the Synthesis of Compounds, 1-15

A mixture of 5.0 mmol of the corresponding ethyl ester **1c**-**15c** in water (50 mL) and tetrahydrofuran (50 mL) and in the presence of potassium hydroxide (1.12 g, 20.0 mmol) was stirred at room temperature for 24 hours. The organic solvent was removed under reduced pressure and the aqueous phase was cooled in an ice bath. After cooling, the pH was adjusted to 2-3 with diluted HCl to obtain the precipitation of the corresponding acid.

*2(R,S)-{2-[(5-Methyl-1*H*-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid (1)*

Trituration with hot AcOEt afforded the titled compound in 85% yield. TLC (AcOEt/MeOH 2:1) – Rf 0.52; mp 275-277 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.39 (s, 3H, -CH_3); 3.16 (m, 2H, -CH_2-); 4.75 (m, 1H, -CH<); 6.86 (s, 1H, Ar); 7.05-7.38 (m, 8H, Ar); 7.48 (s, 1H, Ar); 7.58 (t, 1H, Ar); 7.80 (d, 1H, Ar); 8.62 (d, 1H, Ar); 9.15 (d, 1H, -N<u>H</u>-CH<); 11.80 (s, 1H, -NH-); 12.20 (s, 1H, -NH-); 13.00 (b, 1H, -OH); ^{13}C NMR (DMSO-d_6) δ 21.99, 36.88, 54.94, 102.81, 112.93, 119.83, 120.50, 121.72, 123.19, 126.71, 127.12, 127.87, 128.87, 129.09, 129.51, 129.75, 132.15, 133.31, 136.24, 138.68, 139.97, 159.82, 169.38, 173.41. MS (ES) m/z 442 $[MH]^+$; MW: 441 (calcd. for $C_{26}H_{23}N_3O_4$).

*2(R,S)-{2-[(5-Methoxy-1*H*-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid (2)*

Trituration with hot AcOEt afforded the titled compound in 88% yield. TLC (AcOEt/MeOH 2:1) – Rf 0.45; mp 253-254 °C; ^{1}H NMR (DMSO-d_6) δ 3.20 (m, 2H, -CH_2-); 3.78 (s, 3H, -O-CH_3); 4.74 (m, 1H, -CH<); 6.89 (m, 2H, Ar); 7.10-7.38 (m, 8H, Ar); 7.58 (t, 1H, Ar); 7.81 (d, 1H, Ar); 8.63 (d, 1H, Ar); 9.16 (d, 1H, -N<u>H</u>-CH<); 11.79 (s, 1H, -NH-); 12.25 (s, 1H, -NH-); 13.05 (b, 1H, -OH); ^{13}C NMR (DMSO-d_6) δ 36.86, 54.96, 55.98, 102.73, 103.03, 114.05, 116.20, 119.71,

120.45, 123.14, 127.13, 127.98, 128.90, 129.10, 129.74, 132.45, 133.13, 133.34, 138.70, 140.05, 154.66, 159.73, 169.42, 173.41. MS (ES) m/z 458 $[MH]^+$; MW: 457 (calcd. for $C_{26}H_{23}N_3O_5$).

2(R,S)-{2-[(5,6-Dimethoxy-1*H*-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid (3)

Trituration with hot EtOH afforded the titled compound in 78% yield. TLC (AcOEt/MeOH 2:1) – Rf 0.43; mp 212 °C; ^{1}H NMR (DMSO-d_6) δ 3.23 (m, 2H, -CH_2-); 3.78 (s, 6H, -O-CH_3); 4.73 (m, 1H, -CH<); 6.90 (m, 2H, Ar); 7.10-7.37 (m, 7H, Ar); 7.56 (t, 1H, Ar); 7.79 (d, 1H, Ar); 8.63 (d, 1H, Ar); 9.14 (d, 1H, -N<u>H</u>-CH<); 11.64 (s, 1H, -NH-); 12.16 (s, 1H, -NH-); 13.04 (b, 1H, -OH); ^{13}C NMR (DMSO-d_6) δ 36.85, 54.96, 56.16, 56.37, 95.11, 103.27, 103.64, 119.54, 120.32, 120.53, 122.85, 127.13, 128.91, 129.07, 129.73, 130.38, 132.98, 133.29, 138.71, 140.29, 146.28, 149.58, 159.72, 169.45, 173.41. MS (ES) m/z 488 $[MH]^+$; MW: 487 (calcd. for $C_{27}H_{25}N_3O_6$).

2(R,S)-{2-[(5-Benzyloxy-1*H*-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid (4)

Crystallization from EtOH afforded the titled compound in 85% yield. TLC (AcOEt/MeOH 2:1) – Rf 0.68; mp 258 °C; ^{1}H NMR (DMSO-d_6) δ 3.20 (m, 2H, -CH_2-CH<); 4.74 (m, 1H, -CH<); 5.12 (s, 2H, -O-CH_2-); 6.87 (s, 1H, Ar); 6.96 (m, 1H, Ar); 7.00-7.47 (m, 13H, Ar); 7.58 (t, 1H, Ar); 7.80 (d, 1H, Ar); 8.62 (d, 1H, Ar); 9.15 (d, 1H, -NH-CH<); 11.79 (s, 1H, -NH-); 12.22 (s, 1H, -NH-); 13.00 (b, 1H, -OH); ^{13}C NMR (DMSO-d_6) δ 36.91, 54.96, 70.29, 103.03, 104.26, 114.11, 116.69, 119.77, 120.46, 123.17, 127.12, 127.93, 128.40, 128.90, 129.10, 129.74, 132.55, 133.25, 138.18, 138.69, 140.01, 153.65, 159.69, 169.39, 173.40. MS (ES) m/z 534 $[MH]^+$; MW: 533 (calcd. for $C_{32}H_{27}N_3O_5$).

2(R,S)-{2-[(5-Chloro-1*H*-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid (5)

Crystallization from EtOH afforded the titled compound in 90% yield. TLC (AcOEt/MeOH 2:1) – Rf 0.49; mp 279-280 °C; ^{1}H NMR (DMSO-d_6) δ 3.11 (m, 2H, -CH_2-); 4.74 (m, 1H, -CH<); 6.93 (s, 1H, Ar); 7.07-7.35 (m, 7H, Ar); 7.46 (d, 1H, Ar); 7.58 (t, 1H, Ar); 7.83 (m, 2H, Ar); 8.60 (d, 1H, Ar); 9.17 (d, 1H, -N<u>H</u>-CH<); 12.13 (s, 1H, -NH-); 12.28 (s, 1H, -NH-); 13.04 (b, 1H, -OH); ^{13}C NMR (DMSO-d_6) δ 36.91, 54.94, 102.86, 114.81, 119.96, 120.66, 121.68, 123.44, 124.93, 125.35, 127.10, 128.69, 128.89, 129.09, 129.74, 133.34, 133.65, 136.16, 138.67, 139.76, 159.39, 169.33, 173.36. MS (ES) m/z 462 $[MH]^+$; MW: 461 (calcd. for $C_{25}H_{20}ClN_3O_4$).

2(R,S)-{2-[(5-Nitro-1*H*-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid (6)

Crystallization from EtOH afforded the titled compound in 87% yield. TLC (AcOEt/MeOH 2:1) – Rf 0.47; mp 281 °C; ^{1}H NMR (DMSO-d_6) δ 3.14 (m, 2H, -CH_2-); 4.76 (m, 1H, -CH<); 7.11-7.36 (m, 7H, Ar); 7.62 (m, 2H, Ar); 7.83 (d, 1H, Ar); 8.12 (d, 1H, Ar); 8.58 (d, 1H, Ar); 8.83 (s, 1H, Ar); 9.18 (d, 1H, -N<u>H</u>-CH<); 12.34 (s, 1H, -NH-); 12.64 (s, 1H, -NH-); ^{13}C NMR (DMSO-d_6) δ 36.92, 54.94, 105.78, 113.75, 119.72, 120.26, 120.35, 120.87, 123.73, 126.97, 127.10, 128.89, 129.09, 129.75, 133.35, 135.67, 138.66, 139.49, 140.52, 142.19, 158.95, 169.28, 173.35. MS (ES) m/z 473 $[MH]^+$; MW: 472 (calcd. for $C_{25}H_{20}N_4O_6$).

2(R,S)-{2-[(1-Methyl-5-Nitro-1*H*-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid (7)

Crystallization from EtOH afforded the titled compound in 82% yield. TLC (AcOEt/MeOH 2:1) – Rf 0.63; mp 267-269 °C; ^{1}H NMR (DMSO-d_6) δ 3.19 (m, 2H, -CH_2-); 4.09 (s, 3H, >N-CH_3); 4.73 (m, 1H, -CH<); 7.09-7.36 (m, 7H, Ar); 7.59 (t, 1H, Ar); 7.81 (m, 2H, Ar); 8.17 (m, 1H, Ar); 8.54 (d, 1H, Ar); 8.80 (m, 1H, Ar); 9.17 (d, 1H, -N<u>H</u>-CH<); 12.18 (s, 1H, -NH-); 13.00 (b, 1H, -OH-); ^{13}C NMR (DMSO-d_6) δ 33.03, 36.91, 54.94, 107.67, 112.33, 119.59, 120.12, 120.71, 120.91, 123.85, 125.35, 127.08, 128.88, 129.07, 129.77, 133.21, 136.17, 138.68, 139.38, 142.06, 142.33, 159.63, 169.23, 173.35. MS (ES) m/z 487 $[MH]^+$; MW: 486 (calcd. for $C_{26}H_{22}N_4O_6$).

2(R,S)-{2-[(5-Fluoro-1*H*-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid (8)

Crystallization from EtOH afforded the titled compound in 75% yield. TLC (AcOEt/MeOH 2:1) – Rf 0.42; mp 284-286 °C; ^{1}H NMR (DMSO-d_6) δ 3.23 (m, 2H, -CH_2-); 4.76 (m, 1H, -CH<); 6.95 (s, 1H, Ar); 7.06-7.37 (m, 7H, Ar); 7.43-7.63 (m, 3H, Ar); 7.84 (d, 1H, Ar); 8.62 (d, 1H, Ar); 9.20 (d, 1H, -N<u>H</u>-CH<); 12.05 (s, 1H, -NH-); 12.31 (s, 1H, -NH-); 13.04 (b, 1H, -OH); ^{13}C NMR (DMSO-d_6) δ 36.05, 54.15, 102.41, 105.72, 106.19, 112.56, 113.08, 113.54, 113.74, 119.00, 119.74, 122.57, 126.30, 126.83, 127.03, 128.07, 128.32, 128.94, 132.56, 133.04, 133.72, 137.90, 139.05, 154.82, 158.63, 159.45, 168.53, 172.55. MS (ES) m/z 446 $[MH]^+$; MW: 445 (calcd. for $C_{25}H_{20}FN_3O_4$).

2(R,S)-{2-[(4-Fluoro-1*H*-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid (9)

Crystallization from EtOH afforded the titled compound in 78% yield. TLC (AcOEt/MeOH 2:1) – Rf 0.68; mp 273-275 °C; ^{1}H NMR (DMSO-d_6) δ 3.15 (m, 2H, -CH_2-); 4.79 (m, 1H, -CH<); 6.87 (m, 1H, Ar); 6.95 (s, 1H, Ar); 7.08-7.37 (m, 8H, Ar); 7.60 (t, 1H, Ar); 7.82 (d, 1H, Ar); 8.61 (d, 1H, Ar); 9.19 (d, 1H, -N<u>H</u>-CH<); 12.31 (m, 2H, -NH-); 13.00 (b, 1H, -OH); ^{13}C NMR (DMSO-d_6) δ 36.92, 54.87, 98.72, 105.05, 105.40, 109.82, 116.65, 117.08, 120.13, 120.67, 123.57, 125.41, 125.55, 127.12, 128.86, 129.10, 129.74, 132.73, 133.37, 138.57, 139.61, 139.98, 140.19, 154.27, 159.22, 169.35, 173.34. MS (ES) m/z 446 $[MH]^+$; MW: 445 (calcd. for $C_{25}H_{20}FN_3O_4$).

2(R,S)-{2-[(6-Fluoro-1*H*-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid (10)

Crystallization from EtOH afforded the titled compound in 78% yield. TLC (AcOEt/MeOH 2:1) – Rf 0.66; mp 280 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.20 (m, 2H, -CH_2-); 4.76 (m, 1H, -CH<); 6.98 (m, 2H, Ar); 7.12-7.36 (m, 7H, Ar); 7.58 (t, 1H, Ar); 7.79 (m, 2H, Ar); 8.61 (d, 1H, Ar); 9.17 (d, 1H, -N<u>H</u>-CH<); 11.99 (s, 1H, -NH-); 12.24 (s, 1H, -NH-); 12.95 (b, 1H, -OH); ^{13}C NMR (DMSO-d_6) δ 36.91, 54.92, 98.39, 98.90, 103.60, 109.92, 110.41, 119.89, 120.58, 123.30, 124.16, 124.37, 124.54, 127.12, 128.90, 129.10, 129.75, 132.98, 133.06, 133.34, 137.63, 137.89, 138.68,

139.88, 158.55, 159.47, 163.29, 169.37, 173.38. MS (ES) m/z 446 $[MH]^+$; MW: 445 (calcd. for $C_{25}H_{20}FN_3O_4$).

2(R,S)-{2-[(7-Fluoro-1*H*-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid (11)

Crystallization from EtOH afforded the titled compound in 89% yield. TLC (AcOEt/MeOH 2:1) – Rf 0.66; mp 265 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.15 (m, 2H, -CH_2-); 4.76 (m, 1H, -CH<); 7.02-7.36 (m, 9H, Ar); 7.59 (m, 2H, Ar); 7.81 (d, 1H, Ar); 8.60 (d, 1H, Ar); 9.17 (d, 1H, -NH-CH<); 12.24 (s, 1H, -NH-); 12.42 (s, 1H, -NH-); 13.00 (b, 1H, -OH); ^{13}C NMR (DMSO-d_6) δ 36.91, 54.92, 104.36, 109.20, 109.52, 118.67, 118.73, 120.11, 120.67, 121.15, 121.26, 123.45, 126.04, 126.30, 127.11, 128.88, 129.10, 129.75, 131.31, 131.42, 133.32, 133.79, 138.66, 139.75, 147.51, 152.41, 159.31, 169.34, 173.37. MS (ES) m/z 446 $[MH]^+$; MW: 445 (calcd. for $C_{25}H_{20}FN_3O_4$).

2(R,S)-{2-[(5,7-Difluoro-1*H*-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid (12)

Trituration with hot EtOH afforded the titled compound in 61% yield. TLC (AcOEt/MeOH 2:1) – Rf 0.56; mp 279 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.10 (m, 2H, -CH_2-); 4.76 (m, 1H, -CH<); 7.03 (s, 1H, Ar); 7.11-7.36 (m, 7H, Ar); 7.45 (d, 1H, Ar); 7.60 (t, 1H, Ar); 7.83 (d, 1H, Ar); 8.60 (d, 1H, Ar); 9.19 (d, 1H, -NH-CH<); 12.29 (s, 1H, -NH-); 12.58 (s, 1H, -NH-); 13.03 (b, 1H, -OH); ^{13}C NMR (DMSO-d_6) δ 36.08, 54.05, 98.74, 99.15, 99.35, 99.77, 101.97, 102.06, 102.44, 102.53, 103.68, 119.25, 119.88, 122.13, 122.39, 122.72, 126.27, 128.05, 128.27, 128.90, 129.00, 129.14, 129.25, 129.39, 132.51, 134.37, 137.81, 138.84, 145.82, 146.11, 150.78, 151.07, 153.55, 153.74, 158.17, 158.42, 168.47, 172.49. MS (ES) m/z 464 $[MH]^+$; MW: 463 (calcd. for $C_{25}H_{19}F_2N_3O_4$).

2(R,S)-{2-[(3-Methyl-1*H*-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid (13)

Trituration with hot EtOH abs afforded the titled compound in 80% yield. TLC (AcOEt/MeOH 2:1) – Rf 0.49; mp 247-248 °C; ^{1}H NMR (DMSO-d_6) δ 2.50 (s, 3H, -CH_3); 3.08 (m, 2H, -CH_2-); 4.67 (m, 1H, -CH<); 7.04-7.11 (m, 2H, Ar); 7.17-7.33 (m, 6H, Ar); 7.43 (d, 1H, Ar); 7.57 (t, 1H, Ar); 7.68 (m, 2H, Ar); 8.47 (d, 1H, Ar); 9.11 (d, 1H, -NH-CH<); 11.22 (s, 1H, -NH-); 11.48 (s, 1H, -NH-); 12.91 (b, 1H, -OH); ^{13}C NMR (DMSO-d_6) δ 9.87, 36.28, 54.21, 112.28, 113.08, 119.39, 120.16, 121.24, 122.89, 124.34, 126.42, 128.01, 128.22, 129.10, 132.09, 136.01, 138.01, 138.52, 160.23, 168.38, 172.79. MS (ES) m/z 442 $[MH]^+$; MW: 441 (calcd. for $C_{26}H_{23}N_3O_4$).

2(R,S)-{2-[(7-Methyl-1*H*-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid (14)

Trituration with hot EtOH abs afforded the titled compound in 55% yield. TLC (AcOEt/MeOH 2:1) – Rf 0.54; mp 241 °C; ^{1}H NMR (DMSO-d_6) δ 2.54 (s, 3H, -CH_3); 3.25 (m, 2H, -CH_2-); 4.76 (m, 1H, -CH<); 6.97-7.37 (m, 9H, Ar); 7.54-7.63 (m, 2H, Ar); 7.80 (d, 1H, Ar); 8.65 (d, 1H, Ar); 9.16 (d, 1H, -NH-CH<); 11.76 (s, 1H, -NH-); 12.21 (s, 1H, -NH-); 13.01 (b, 1H, -OH); ^{13}C NMR (DMSO-d_6) δ 17.03, 36.03, 53.97, 103.33, 119.07, 119.19, 119.60, 120.25, 121.91, 122.26, 124.33, 126.18, 126.57, 127.95, 128.17, 128.81, 131.52, 132.32, 136.66, 137.75, 139.07, 158.97, 168.47, 172.40. MS (ES) m/z 442 $[MH]^+$; MW: 441 (calcd. for $C_{26}H_{23}N_3O_4$).

3-Phenyl-2(R,S)-{2-[(7-Trifluoromethyl-1*H*-Indole-2-Carbonyl)-Amino]-Benzoylamino}-Propionic Acid (15)

Trituration with hot EtOH abs afforded the titled compound in 45% yield. TLC (AcOEt/MeOH 2:1) – Rf 0.49; mp 257 °C; ^{1}H NMR (DMSO-d_6) δ 3.21 (m, 2H, -CH_2-); 4.72 (m, 1H, -CH<); 7.04-7.32 (m, 8H, Ar); 7.59 (m, 2H, Ar); 7.78 (d, 1H, Ar); 8.05 (d, 1H, Ar); 8.54 (d, 1H, Ar); 9.12 (d, 1H, -NH-CH<); 11.88 (s, 1H, -NH-); 12.17 (s, 1H, -NH-); 12.95 (b, 1H, -OH); ^{13}C NMR (DMSO-d_6) δ 36.10, 54.05, 104.07, 112.31, 112.96, 113.61, 119.62, 119.69, 119.99, 121.26, 121.62, 122.79, 126.24, 126.71, 128.02, 128.27, 128.74, 128.89, 131.57, 132.43, 133.85, 137.80, 138.74, 158.29, 168.40, 172.48. MS (ES) m/z 496 $[MH]^+$; MW: 495 (calcd. for $C_{26}H_{20}F_3N_3O_4$).

General Procedure for the Synthesis of Compounds, 16-21

A mixture of 0.60 mmol of the corresponding ethyl ester (compounds **16c-21c**) in water (25 mL) and tetrahydrofuran (25 mL) and in the presence of lithium hydroxide monohydrate (25 mg, 0.60 mmol) was stirred at room temperature for 24 hours. The organic solvent was removed under reduced pressure and 30 mL of saturated $NaHCO_3$ solution and 30 mL of AcOEt were added. After a vigorous stirring for a few minutes, the precipitated salt was collected by filtration. The obtained salt was taken up with 30 mL of tetrahydrofuran and the solution was acidified with N HCl. The organic phase was removed under reduced pressure and the product was filtered. Trituration in succession with hot AcOEt and then with absolute EtOH afforded the analytically pure compounds, **16-21**.

2(S)-{2-[(1*H*-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid (16)

Yield: 50%. TLC (AcOEt/MeOH 2:1) – Rf 0.61; mp 270-271 °C; ^{1}H NMR (DMSO-d_6) δ 3.16 (m, 2H, -CH$_2$-CH<); 4.77 (m, 1H, -CH<); 6.95 (s, 1H, H ind); 7.06-7.37 (m, 8H, Ar); 7.47 (d, 1H, Ar); 7.59 (t, 1H, Ar); 7.73 (d, 1H, Ar); 7.81 (d, 1H, Ar); 8.63 (d, 1H, Ar); 9.17 (d, 1H, -NH-CH<); 11.92 (s, 1H, -NH- ind); 12.26 (s, 1H, -NH-); 12.97 (b, 1H, -OH); ^{13}C NMR (DMSO-d_6) δ 36.91, 54.89, 103.32, 113.20, 119.87, 120.54, 120.91, 122.57, 123.23, 124.78, 127.11, 127.65, 128.89, 129.10, 129.74, 132.22, 133.31, 137.80, 138.66, 139.95, 159.77, 169.39, 173.36. MS (ES) *m/z* 428 $[MH]^+$; MW: 427 (calcd. for $C_{25}H_{21}N_3O_4$). $[a]_D^{25}$ -15.8 (*c* 0.57, DMF). Optical Purity [EHPLC]: *e.e.* > 99.5%. Analytical conditions: column CSP-TE-SP-100/5 (250 x 4.0, L x ID mm); detector: UV at 254 nm; eluent: MeOH/H_2O (85/15) 20 mM NH_4OAc; flow rate: 1.00 ml/min; temperature: 23° C; chromatographic parameters: k'_1: 0.61 (t_r: 4.0 min), $\alpha_{R,S}$: 2.33.

2 (R) -{2-[(1*H*-Indole-2-Carbonyl)-Amino]-Benzoylamino}-3-Phenyl-Propionic Acid (17)

Yield: 43%. TLC (AcOEt/MeOH 2:1) – Rf 0.61; mp 271-272 °C; ^{1}H NMR (DMSO-d_6) δ 3.16 (m, 2H, -CH$_2$-CH<);

4.76 (m, 1H, -CH<); 6.95 (s, 1H, H ind); 7.06-7.37 (m, 8H, Ar); 7.47 (d, 1H, Ar); 7.59 (t, 1H, Ar); 7.73 (d, 1H, Ar); 7.81 (d, 1H, Ar); 8.63 (d, 1H, Ar); 9.17 (d, 1H, -NH-CH<); 11.92 (s, 1H, -NH- ind); 12.25 (s, 1H, -NH-); 13.00 (b, 1H, -OH); ^{13}C NMR (DMSO-d_6) δ 36.89, 54.90, 103.31, 113.21, 119.83, 120.53, 120.91, 122.58, 123.24, 124.80, 127.12, 127.64, 128.89, 129.10, 129.75, 132.21, 133.34, 137.80, 138.67, 139.96, 159.77, 169.39, 173.38. MS (ES) *m/z* 428 $[MH]^+$; MW: 427 (calcd. for $C_{25}H_{21}N_3O_4$). $[a]_D^{25}$ +13.6 (*c* 0.59, DMF). Optical Purity [EHPLC]: *e.e.* 98.7%. Analytical conditions: column CSP-TE-SP-100/5 (250 x 4.0, L x ID mm); detector: UV at 254 nm; eluent: MeOH/H_2O (85/15) 20 mM NH_4OAc; flow rate: 1.00 ml/min; temperature: 23° C; chromatographic parameters: k'_2: 1.42 (t_r: 5.6 min), $\alpha_{R,S}$: 2.33.

2(S)-{2-[(5-Fluoro-1H-Indole-2-Carbonyl)-Amino]-Benzo-ylamino}-3-Phenyl-Propionic Acid (18)

Yield: 24%. TLC (AcOEt/MeOH 2:1) – Rf 0.42; mp 283-284 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.25 (m, 2H, -CH$_2$-CH<); 4.76 (m, 1H, -CH<); 6.95 (s, 1H, Ar); 7.08-7.63 (m, 10H, Ar); 7.82 (d, 1H, Ar); 8.62 (d, 1H, Ar); 9.17 (d, 1H, -NH-CH<); 12.03 (s, 1H, -NH- ind); 12.29 (s, 1H, -NH-); 13.08 (b, 1H, -OH); ^{13}C NMR (DMSO-d_6) δ 36.06, 54.07, 102.39, 105.68, 106.14, 112.52, 113.05, 113.51, 113.70, 119.04, 119.76, 122.53, 126.26, 126.80, 127.02, 128.04, 128.25, 128.89, 132.50, 133.02, 133.69, 137.82, 139.00, 154.79, 158.61, 159.43, 168.50, 172.49. MS (ES) m/z 446 $[MH]^+$; MW: 445 (calcd. for $C_{25}H_{20}FN_3O_4$). $[a]_D^{25}$ -14.5 (*c* 0.58, DMF). Optical Purity [EHPLC]: *e.e.* 99.6%. Analytical conditions: column CSP-TE-SP-100/5 (250 x 4.0, L x ID mm); detector: UV at 254 nm; eluent: MeOH/H_2O (85/15) 20 mM NH_4OAc; flow rate: 1.00 ml/min; temperature: 20° C; chromato-graphic parameters: k'_1: 0.47 (t_r: 3.6 min), $\alpha_{R,S}$: 2.65.

2(R)-{2-[(5-Fluoro-1H-Indole-2-Carbonyl)-Amino]-Benzo-ylamino}-3-Phenyl-Propionic Acid (19)

Yield: 49%. TLC (AcOEt/MeOH 2:1) – Rf 0.42; mp 283-284 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.26 (m, 2H, -CH$_2$-CH<); 4.77 (m, 1H, -CH<); 6.96 (s, 1H, Ar); 7.08-7.64 (m, 10H, Ar); 7.83 (d, 1H, Ar); 8.63 (d, 1H, Ar); 9.19 (d, 1H, -NH-CH<); 12.04 (s, 1H, -NH- ind); 12.30 (s, 1H, -NH-); 13.02 (b, 1H, -OH); ^{13}C NMR (DMSO-d_6) δ 36.07, 54.07, 102.40, 102.51, 105.71, 106.17, 112.54, 113.08, 113.53, 113.72, 119.03, 119.77, 122.54, 126.29, 126.83, 127.04, 128.07, 128.28, 128.91, 132.54, 133.04, 133.72, 137.82, 139.04, 154.82, 158.63, 159.46, 168.54, 172.54. MS (ES) m/z 446 $[MH]^+$; MW: 445 (calcd. for $C_{25}H_{20}FN_3O_4$). $[a]_D^{25}$ +13.6 (*c* 0.56, DMF). Optical Purity [EHPLC]: *e.e*. 98.6%. Analytical conditions: column CSP-TE-SP-100/5 (250 x 4.0, L x ID mm); detector: UV at 254 nm; eluent: MeOH/H_2O (85/15) 20 mM NH_4OAc; flow rate: 1.00 ml/min; temperature: 20° C; chromato-graphic parameters: k'_2: 1.24 (t_r: 5.6 min), $\alpha_{R,S}$: 2.65.

2(S)-{2-[(7-Fluoro-1H-Indole-2-Carbonyl)-Amino]-Benzo-ylamino}-3-Phenyl-Propionic Acid (20)

Yield: 62%. TLC (AcOEt/MeOH 2:1) – Rf 0.48; mp 251-252 °C; ^{1}H NMR (DMSO-d_6) δ 3.24 (m, 2H, -CH_2-); 4.75 (m, 1H, -CH<); 7.03-7.35 (m, 9H, Ar); 7.54-7.62 (m, 2H, Ar); 7.81 (d, 1H, Ar); 8.59 (d, 1H, Ar); 9.15 (d, 1H, -NH-CH<); 12.22 (s, 1H, -NH-); 12.40 (s, 1H, -NH-); 13.00 (b, 1H, -OH); ^{13}C NMR (DMSO-d_6) δ 36.10, 54.05, 103.52, 108.35, 108.66, 117.88, 119.33, 119.84, 120.30, 120.42, 122.60, 125.20, 125.47, 126.26, 128.03, 128.25, 128.90, 130.48, 130.59, 132.44, 132.96, 137.81, 138.91, 146.68, 151.57, 158.47, 168.49, 172.49. MS (ES) m/z 446 $[MH]^+$; MW: 445 (calcd. for $C_{25}H_{20}FN_3O_4$). $[a]_D^{25}$ -17.8 (*c* 0.67, DMF). Optical Purity [EHPLC]: *e.e.* >99.9%. Analytical conditions: column CSP-TE-SP-100/5 (250 x 4.0, L x ID mm); detector: UV at 254 nm; eluent: MeOH/H_2O (85/15) 20 mM NH_4OAc; flow rate: 1.00 ml/min; temperature: 20° C; chromatographic parameters: k'_1: 0.49 (t_r: 3.6 min), $\alpha_{R,S}$: 2.52.

2(R)-{2-[(7-Fluoro-1H-Indole-2-Carbonyl)-Amino]-Benzo-ylamino}-3-Phenyl-Propionic Acid (21)

Yield: 64%. TLC (AcOEt/MeOH 2:1) – Rf 0.48; mp 252 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.25 (m, 2H, -CH_2-CH<); 4.75 (m, 1H, -CH<); 7.03-7.35 (m, 9H, Ar); 7.55-7.62 (m, 2H, Ar); 7.81 (d, 1H, Ar); 8.59 (d, 1H, Ar); 9.15 (d, 1H, -NH-CH<); 12.22 (s, 1H, -NH-); 12.41 (s, 1H, -NH-); 13.00 (s, 1H, -OH); ^{13}C NMR (DMSO-d_6) δ 36.09, 54.06, 103.53, 108.33, 108.65, 117.80, 119.36, 119.84, 120.28, 122.59, 125.19, 125.46, 126.24, 128.01, 128.23, 128.88, 130.47, 130.58, 132.42, 132.95, 137.82, 138.88, 146.67, 151.56, 158.47, 168.47, 172.47. MS (ES) m/z 446 $[MH]^+$; MW: 445 (calcd. for $C_{25}H_{20}FN_3O_4$). $[a]_D^{25}$ +14.4 (*c* 0.77, DMF). Optical Purity [EHPLC]: *e.e.* 97.7%. Analytical conditions: column CSP-TE-SP-100/5 (250 x 4.0, L x ID mm); detector: UV at 254 nm; eluent: MeOH/H_2O (85/15) 20 mM NH_4OAc; flow rate: 1.00 ml/min; temperature: 20° C; chromatographic parameters: k'_2: 1.23 (t_r: 5.4 min), $\alpha_{R,S}$: 2.52.

Biological Evaluations

Male Hartley guinea pigs (300-350 g) and male Sprague Dawley rats (250-300 g) were used. For binding assays to isolated rat pancreatic acini, animals were fasted, but allowed free access to water, for 18 - 24 h prior to the experiment.

[^{125}I]-BH-CCK-8 (CCK_8(sulphated), [^{125}I]Bolton and Hunter labeled-specific activity 2000 Ci/mol) was purchased from Amersham Pharmacia Biotech (Bucking-hamshire, UK). All other drugs and reagents were obtained from commercial sources.

The binding parameters for the substances under investigation, IC_{50} values and standard errors were calculated from concentration-response curve analyzed by a computerized curve fitting technique (ALLFIT) using the four parameter logistic equation [36].

[^{125}I]BH-CCK-8 Receptor Binding Assay in Isolated Rat Pancreatic Acinar Cells

Isolated pancreatic acini were prepared by enzymatic digestion of pancreas as previously described by Makovec F. *et al*. [17]. Drug displacing experiments were carried out by incubating acinar cells, [^{125}I]BH-CCK-8 (25 pM final concentration) and competitors in 0.5 mL total volume at 37 °C for 30 min, in shaking bath. At the end of incubation, 1 mL of ice-cold Hepes-Ringer buffer (10 mM Hepes, 118 mM NaCl, 1.13 mM $MgCl_2$, 1.28 $CaCl_2$, 1% BSA, 0.2 mg/mL Soybean trypsin inhibitor, pH 7.4) was added and the tubes were centrifuged 5 min at 12500g. The supernatant was aspirated and the radioactivity associated to the pellet measured. The non-specific binding was estimated in the presence of 1 µM CCK-8, accounting 15% of total binding.

[^{125}I]BH-CCK-8 Receptor Binding Assay in Guinea Pig Cerebral Cortices

Membranes from guinea pig cerebral cortices were prepared as previously described [17]. Protein concentration was determined according to Bradford [37], using bovine serum albumin (BSA) as standard. The binding experiments were performed in assay buffer containing 10 mM Hepes, 118 mM NaCl, 4.7 mM KCl, 5.0 mM $MgCl_2$, 1.0 mM EGTA, pH 6.5 and supplemented with 0.2 mg/mL bacitracin. The incubation of membranes suspension with labeled ligand and inhibitors was carried out in a microtiter 96-wells filter plate (Multiscreen, Millipore Inc, Bedford, MA) with integral Whatman GF/B membrane filters. Aliquot of membranes (0.5 mg of protein/mL) were added to each well, containing [^{125}I]BH-CCK8 (25 pM), in a final volume of 250 µl. The non-specific binding of iodinated peptide was defined in the presence of 1µM CCK-8, accounting for 20% of total binding. Nonspecific binding of [^{125}I]BH-CCK-8 to membrane filters (blank), measured in wells containing an equal amount of labeled ligand, but no membranes, was 0.2% of total radioligand added. After 120 min at 25 °C, the 96-wells plate was placed on the vacuum filtration apparatus (Millipore Inc.). The integral membrane filters were rinsed with 0.25 mL of ice cold assay buffer, dried, punched into polycarbonate tubes and counted in a COBRA-5002 γ-counter (Packard Biosciences).

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