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Anthranilic acid based CCK_1 receptor antagonists: Blocking the receptor with the same 'words' of the endogenous ligand

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ABSTRACT

The anthranilic acid diamides represent the more recent class of nonpeptide CCK_1 receptor antagonists. This class is characterized by the presence of anthranilic acid, used as a molecular scaffold, and two pharmacophores selected from the C-terminal tetrapeptide of CCK. The lead compound coded VL-0395, endowed with sub-micromolar affinity towards CCK_1 receptors, was characterized by the presence of Phe and 2-indole moiety at the C- and N-termini of anthranilic acid, respectively. Herein we describe the first step of the anthranilic acid C-terminal optimization using, instead of Phe, aminoacids belonging to the primary structure of CCK-8 and other not coded residues. Thus we demonstrate that the CCK_1 receptor affinity depends on the nature of the aminoacidic side chain as well as that the free carboxy group of the alpha-aminoacids is crucial for the binding. The R enantiomers of the most active compounds represent the eutomers of this class of antagonists confirming thus the stereo preference of the receptor. Moreover this SAR study demonstrates that the receptor binding pocket, that host the aminoacidic side chain, results much more tolerant respect to that accommodating the indole ring. As a result, an appropriate variation of the aminoacidic side chain could provide a better CCK_1 receptor affinity diorthosis.

1. Introduction

Cholecystokinin (CCK) is a gut-brain peptide which acts as hormone in the gastrointestinal (GI) tract and as neurotransmitter/neuromodulator at the central nervous system (CNS).[1–4] The entire range of peripheral and central biological actions of CCK is mediated by two distinct G-protein coupled receptor types: CCK_1 and CCK_2.[5–8] Among the different molecular forms of CCK only the C-terminal octapeptide (Asp[26]-Tyr(SO_3H)[27]-Met[28]-Gly[29]-Trp[30]-Met[31]-Asp[32]-Phe[33]-NH_2 or CCK-8S) binds both receptors with nanomolar affinity. The smallest bioactive fragment of CCK (Trp[30]-Met[31]-Asp[32]-Phe[33]-NH_2 or CCK-4) binds with nanomolar affinity only the CCK_2 receptor while its affinity towards the CCK_1 receptor decreases to the millimolar range.[9]

In view of the importance of CCK in different physiopathological processes as irritable bowel syndrome (IBS), chronic and acute pancreatitis, gastric ulcer, anxiety, panic disorder, eating disorders etc., over the past 15 years, great efforts have been made to develop clinically useful small non-peptide molecules known as CCK receptor antagonists.[10–15]

Among the strategies adopted for the design of these antagonists, a particular consideration was given to the following four approaches: (a) The chemical simplification and manipulation of natural products, such as asperlicin[16,17] (Fig. 1); (b) The dipeptoid approach, in which the minimal structure responsible for the binding is a dipeptide derived from the C-terminal sequence of CCK; (c) The key aminoacid derivatization, in which the receptor recognition is assigned to a single aminoacid;[18–20] and (d) The use of molecular scaffolds bearing suitable pharmacophoric groups.[21–24] All these strategies gave rise to a variety of CCK_1 receptor antagonists characterized by different structure and structural complexity, reflecting thus the philosophy of the designer, as well as by a dissimilar biological profile.

In an earlier report, we described 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 (CCK-4) and by the anthranilic acid dimer used as molecular scaffold.[23,24]

The lead compound obtained from this part of our work coded **VL-0494** (Fig. 1) resembles some structural features of CCK-4

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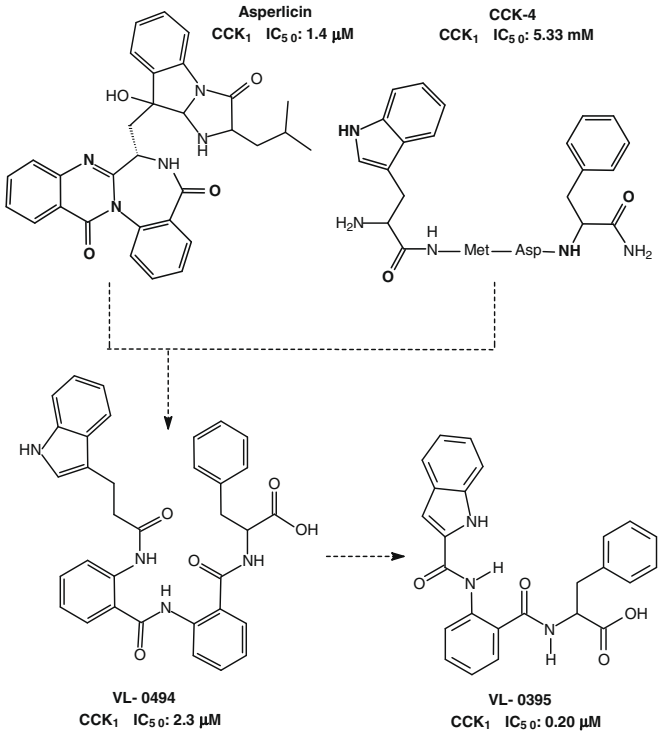


Figure 1. Chemical structure and CCK_1 receptor affinity of Asperlicin, CCK-4, VL-0494 and VL-0395.

having these two 'tetrapeptides' the same N- and C-terminal aminoacid side chains.[25]

Further simplification of anthranilic acid dimer scaffold to a monomer led to a compound coded **VL-0395** (Fig. 1) endowed with submicromolar affinity (IC_{50} = 197,5 nM) representing the new starting point for the development of this innovative class of CCK_1 receptor antagonists.[26] The antagonist nature of **VL-0395** was confirmed by an in vivo functional test. The compound inhibited the guinea pig gallbladder contractions, induced by CCK-8, with a potency comparable to that of the reference CCK_1 selective antagonist Loxiglumide.[26]

It is interesting to notice that both the lead compounds **VL-0494** and **VL-0395** share at the C-terminal site the Phe residue utilized in our design strategy for its 'address' type contribute according to the 'message and address' theory described by Schwyzer.[27]

So, in order to initiate the second phase of the development of this new class of CCK_1 receptor antagonists, regarding the C-terminal optimization of the anthranilic acid of **VL-0395,** we propose to synthesize the compounds reported in Table 1 and test their affinity towards CCK receptors.

First of all, we select to introduce, on the C-terminus of anthranilic acid, aminoacids belonging to CCK-8, that is, Gly, Met, Asp, Trp, and Tyr (Compounds **1**, **5**, **7**, **9** and **10**, respectively). In addition to the above single components of CCK-8 we select other not coded aminoacids such as Ala, Val, Leu and Glu (Compounds **2**, **3**, **4**, and **8**, respectively) for the following reasons. Alanine, characterized by the simplest side chain, is selected in order to ascertain the relative importance of the other aminoacids. Valine and leucine are proposed in order to establish the importance of branched aliphatic side chains while the glutamic acid because has provided an important class of ligands for both CCK receptors subtypes.[28,29]

Secondly, compounds **12–21** are proposed in order to elucidate the CCK_1 receptor binding role of the C-terminal free carboxy group of Phe. It is interesting to notice that the C-terminal amide group of CCK is crucial for the full biological activity and that CCK analogs lacking the C-terminal primary amide group were able to antagonize the contractile activity induced by CCK-7 or CCK-8.[30] Moreover, the same kind of modifications applied to the C-terminal tetrapeptide of CCK, produced potent gastrin antagonists (e.g., Boc-Trp-Leu-Asp-2-phenylethylamide, Boc-Trp-Leu-Asp-2-phenylethyl ester).[31]

Finally, the pure enantiomers (compounds **22–25**) of the most active compounds have been synthesized and tested in order to confirm the stereo preference of the receptor.

2. Chemistry

The procedure for the synthesis of aminoacidic derivatives **1–11** and **19–25** is depicted in Scheme 1.

DL-Aminoacids were protected by conversion to ethyl esters **1a–5a**, **7a–9a**, **14a** and **19a–21a**. The optically active aminoacids were purchased as ethyl esters hydrochlorides. For the tyrosine derivative **11a**, both carboxylic and phenolic groups were protected by benzylation at carboxy and hydroxy groups, following a described procedure.[32] Subsequently deprotection of the amino group by treatment with gaseous HCl in ethyl acetate let us to obtain compound **11a**.

The *o*-aminobenzoylation of DL, L or D-aminoacid ethyl esters by isatoic anhydride gave compounds **1b–5b**, **7b–9b**, **11b**, **14b** and **19b–25b**, several of which previously described.[23]

For compound **8b** the synthesis of the *o*-nitrobenzoyl derivative was preferred, followed by reduction of the nitro group.

The *o*-aminobenzoyl derivatives were converted to compounds **1c–9c**, **11c**, **14**, and **19c–25c** by reaction with indole-2-carboxylic acid or *N*-methyl-indole-2-carboxylic acid activated by treatment with PCl_5. The last step concerning the deprotection of the carboxylic group of intermediates **1c–9c**, **11c**, and **19c–21c** by base catalyzed hydrolysis, gave final compounds **1–9**, **11** and **19–25**.

The optical purity of the enantiomers (compounds **22–25**) was determined by analytical enantioselective HPLC (EHPLC) under reverse phase conditions.

Compound **11** was converted to derivative **10** by deprotection of the phenolic group by catalytic hydrogenation.

The strategy for the synthesis of amine derivatives **16–18** is identical, following the same procedures to convert amphetamine, 2-phenylethylamine and tryptamine to compounds **16–18** respectively, without protection (Scheme 2).

The latter compounds (**12**, **13** and **15**) were obtained with different procedures (Scheme 3).

The ethyl ester **14** was treated with a solution of methanol saturated with gaseous ammonia to give the amide **12**.

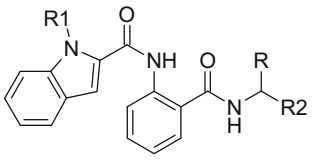
The lead compound **VL-0395** was activated as mixed anhydride with isobutyl chloroformate or ethyl chloroformate to give, by treatment with dipentylamine, the amide derivative **13** and, by reduction with $NaBH_4$, the corresponding alcohol **15**.[33]

3. Biological activity

Binding data, along with those of the lead **VL-0395**, are reported in Table 1. The obtained affinities for CCK_1 and CCK_2 receptors of the synthesised compounds **1–25** were evaluated according to established protocols and are expressed as IC_{50} or as percentage of inhibition (ISB%) determined at the highest used dose (1 μM, 3 μM, 10 μM or 30 μM as indicated).[20] When the ISB% values were lower than 20% the compounds were considered inactive. Values without standard errors were obtained from no more than two experiments.

The present compounds can be divided into three groups (Table 1) and will be discussed separately according to the prefixed tar-

Table 1
CCK receptors binding data



Compd	R	R1	R2	Stereo	IC_{50} (μM)[a]	
					Rat pancreatic acini (CCK_1)	Guinea pig brain cortex (CCK_2[f])
VL-0395	$-CH_2-C_6H_5$	H	–COOH	RS	0.197 ± 0.107	16.40
1	–H	H	–COOH	RS	5.52 ± 1.50	IN[d]
2	$-CH_3$	H	–COOH	RS	3.24 ± 1.18	IN[d]
3	$-CH-(CH_3)_2$	H	–COOH	RS	0.288 ± 0.070	27.40
4	$-CH_2-CH-(CH_3)_2$	H	–COOH	RS	0.083 ± 0.008	IN[d]
5	$-(CH_2)_2-S-CH_3$	H	–COOH	RS	0.052 ± 0.007	IN[d]
6	$-(CH_2)_2-S-CH_3$	CH_3	–COOH	RS	0.092 ± 0.006	IN[d]
7	$-CH_2-COOH$	H	–COOH	RS	IN[d]	36% ISB[b]
8	$-(CH_2)_2-COOH$	H	–COOH	RS	IN[d]	IN[d]
9	$-CH_2$-3-indolyl	H	–COOH	RS	0.445 ± 0.160	IN[d]
10	$-CH_2-C_6H_4$-4-OH	H	–COOH	RS	2.065 ± 0.576	IN[d]
11	$-CH_2-C_6H_4$-4-$OCH_2C_6H_5$	H	–COOH	RS	1.158 ± 0.408	IN[d]
12	$-CH_2-C_6H_5$	H	$-CONH_2$	RS	8.530 ± 3.18	IN[d]
13	$-CH_2-C_6H_5$	H	$-CON(C_5H_{11})_2$	RS	IN[c]	IN[e]
14	$-CH_2-C_6H_5$	H	$-COOCH_2CH_3$	RS	20% ISB[c]	IN[e]
15	$-CH_2-C_6H_5$	H	$-CH_2OH$	RS	32% ISB[c]	NT
16	$-CH_2-C_6H_5$	H	$-CH_3$	RS	3.15 ± 0.27	IN[d]
17	$-CH_2-C_6H_5$	H	–H	–	IN[c]	IN[d]
18	$-CH_2$-3-indolyl	H	–H	–	IN[c]	IN[d]
19	–H	H	$-CH_2COOH$	–	IN[c]	IN[e]
20	–H	H	$-(CH_2)_2COOH$	–	IN[c]	IN[e]
21	$-CH_2-C_6H_5$	H	$-CH_2COOH$	RS	25% ISB[d]	16.90
22	$-CH_2-CH-(CH_3)_2$	H	–COOH	S	4.64 ± 0.110	45% ISB[e]
23	$-CH_2-CH-(CH_3)_2$	H	–COOH	R	0.046 ± 0.004	14.7 ± 1.2
24	$-(CH_2)_2-S-CH_3$	H	–COOH	S	2.75 ± 0.093	31% ISB[e]
25	$-(CH_2)_2-S-CH_3$	H	–COOH	R	0.027 ± 0.002	11.4 ± 0.8

IN: inactive (ISB less than 20%). NT: not tested.
[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.
[e] 30 μM.
[f] Values without standard errors were obtained from not more than two experiments.

gets (importance of the aminoacid side chain, of C-terminal free carboxy group and that of the chiral center configuration).

4. Results and discussion

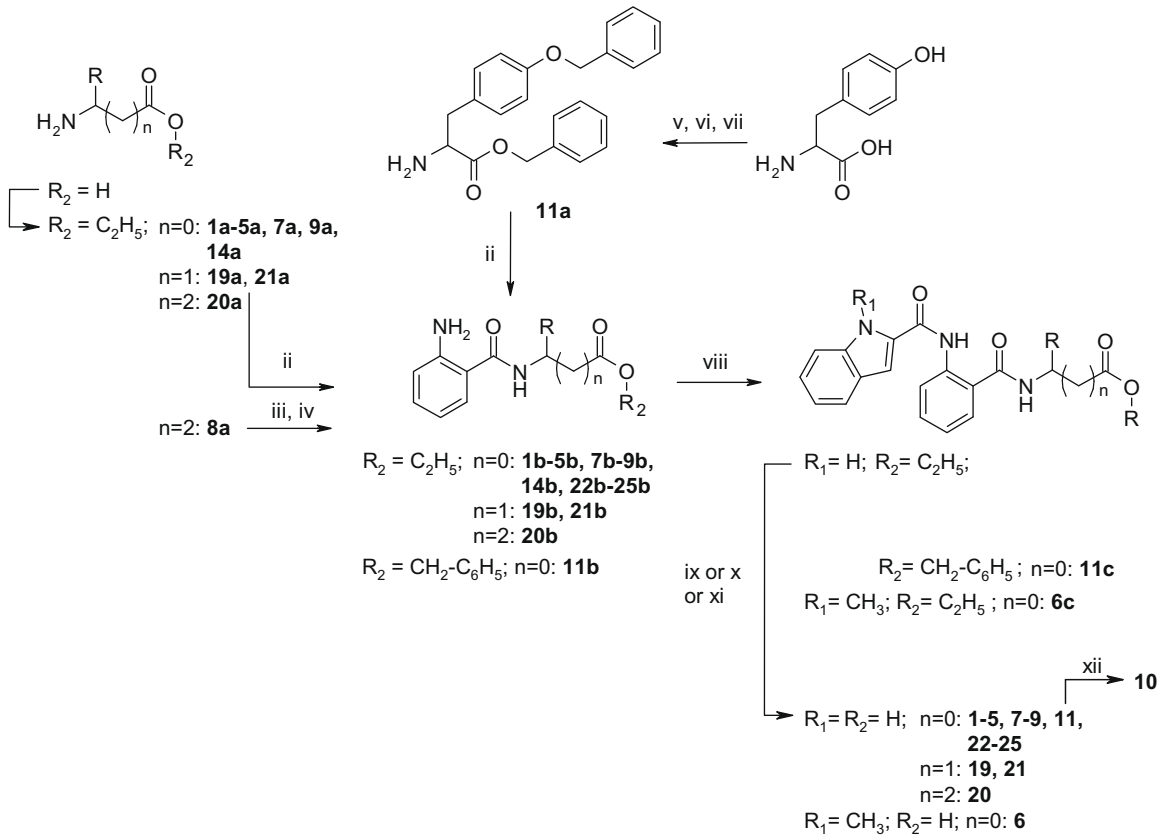
4.1. SAR of the side chain

As previously observed,[26] all the tested compounds showed low affinity towards the central CCK_2 receptor subtype confirming their preference for the CCK_1 receptors.

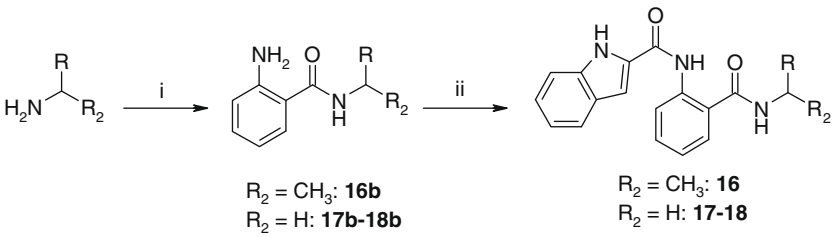
A macroscopic analysis of the obtained data shows that the binding affinity of compounds **1–11** appears clearly influenced by the nature of the aminoacidic side chain. In fact, the lowest affinity observed was that of the Gly derivative (**1**) which is devoid of side chain, thus offering a major conformational flexibility to the C-terminal carboxy group. The Ala derivative (**2**) resulted two fold more potent than Gly and its affinity value is useful as reference in this SAR study dedicated to the side chain optimization. Substitution of the methyl group of Ala (**2**) with the *iso*-propyl group of Val (**3**) enhance ten fold the CCK_1 receptor affinity. This compound showed an affinity and apparent selectivity comparable to that of the reference compound **VL-0395** bearing the Phe residue. Moreover, further improvement in affinity was obtained when the *iso*-propyl group was distanced from the aminoacidic backbone by a methylene unit as in the Leu derivative (**4**). In this case the *iso*-propyl group of Leu can be viewed as bioisoster of the phenyl group of Phe. Nevertheless, although the gain in affinity observed with the Leu derivative characterized by the presence of aliphatic branched side chain, the best result in binding affinity in this series was obtained by the linear chain of Met (**5**). The four fold enhancement in affinity of the Met derivative respect to that of **VL-0395** suggests that a correct interaction with the hydrophobic binding pocket of the receptor can be established not only by the aromatic ring of Phe but even by linear aliphatic chains. Therefore, this receptor pocket that host the aminoacidic side chain seems to be much more tolerant respect to that accommodating the indole ring which imposes a high degree of conformational restrictions.[34] Indeed, the introduction of a methyl group at the indole nitrogen of **5** (**6**) produce a slight drop in affinity similar to that observed for the *N*-methyl indole derivative of **VL-0395**.[35] This fact suggests that the Met derivative interacts with the same receptor binding pockets of **VL-0395**.

Furthermore, the loss in affinity showed by the hydrophilic chains of Asp and Glu confirms the hydrophobic nature of the receptor pocket.

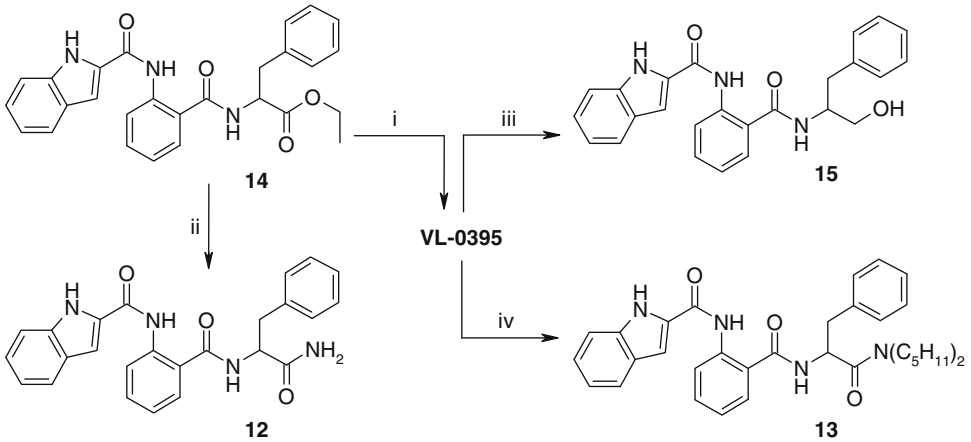
The large aminoacid Trp of **9** seems to reach the binding pocket but its lowered affinity, possibly due to the steric hindrance of the indole system, underlines a non optimal fitting with the receptor sub-site.



Scheme 1. Synthesis of compounds **1**–**11, 19**–**25**. Reagents and conditions: (i) HCl (g), EtOH abs.; (ii) Isatoic anhydride, TEA, EtOAc, reflux; (iii) *o*-nitrobenzoyl chloride, Et_3N, CH_2Cl_2; (iv) Zn, AcOH, CH_2Cl_2 ; (v) $(Boc)_2O$, dioxane, NaOH 1 N, H_2O; (vi) benzyl bromide, K_2CO_3, acetone reflux; (vii) HCl (g), EtOAc; (viii) indole-2-carboxylic acid or *N*-methyl-indol-2-carboxylic acid, PCl_5, dry CH_2Cl_2, pyridine or TEA; (ix) KOH, THF/H_2O 1:1; (x) KOH, MeOH, reflux; (xi) LiOH, THF/H_2O 1:1; (xii) H_2, 10% Pd/C, THF.



Scheme 2. Synthesis of compounds **16**–**18**. Reagents and conditions: (i) isatoic anhydride, EtOAc, reflux; (ii) indol-2-carboxylic acid, PCl_5, dry CH_2Cl_2, pyridine or TEA.



Scheme 3. Synthesis of compounds **12**, **13, 15**. Reagents and conditions: (i) KOH, THF/H_2O 1:1; (ii) NH_3 (g), MeOH; (iii) EtOCOCl, *N*-methylmorpholine, THF, DMF, $NaBH_4$, MeOH; (iv) $NH(C_5H_{11})_2$, ^{i}BuOCOCl, TEA, THF.

The Tyr derivative **10** deserves a particular attention because represent a key aminoacid of CCK-8 in its recognition approach.[36] The affinity observed for the Tyr derivative **10** was ten fold lower respect **VL-0395** and similar to that of Ala (**2**). Even the Tyr O-protected derivative (**11**) resulted more active than compound **10**.

In any case, we are not surprised by this result since a similar behavior was already observed in a previous SAR study concerning the substitution of the Phe phenyl ring. In particular, all the four substituted derivatives of Phe were from two to sixfold less active than **VL-0395** whereas the 2 and 3 substituted compounds were active as the lead.[37]

The antagonist nature of the most active compounds **4** and **5** was confirmed by an in vitro functional test on isolated muscular strips of guinea pig gallbladder stimulated by CCK-8. Both compounds showed a 100% inhibition at the maximum concentration tested of 1 μM.

Moreover, trying to correlate the observed affinities of compounds **1–11** to the hydrophobic parameter *C*log*P* (calculated by the CHEMDRAW ULTRA 7.01 Software) it appears that the highest affinities are related to *C*log*P* range of 2.5–4.

4.2. Molecular modeling

In order to evaluate the spatial arrangement of the pharmacophoric groups of the most active compounds a conformational analysis was carried out on compounds **4** and **5**, and their geometries were compared with that of the lead **VL-0395**. As to the structure of the lead, we started from the NMR-derived conformation that has been recently identified.[36] The structure of **VL-0395** was built manually in this conformation and then the system was optimized by an AM1 calculation. We had already followed this procedure in the past for the same compound in its neutral and protonated forms.[35] Here the optimization was repeated on the carboxylic anion of **VL-0395**, as in the NMR derived structure and a Monte Carlo conformational search was performed. The search yielded 142 conformations for the anion of **VL-0395**, but none was lower in energy than the NMR-derived structure (Fig. 2a). The geometries of the anions **4** and **5** were then built over the minimum energy conformation of **VL-0395**, and both the molecules were submitted to a conformational search. The searches gave this time rather different results, and the geometry corresponding to the NMR minimum of **VL-0395** is not the absolute minimum for both Leu and Met derivatives (Figs. 2b and c).

The enthalpy of formation for the minimum energy conformation of compound **4** is 2.1 kcal/mol more favorable than that with the backbone in the same conformation of **VL-0395**. This geometry is by the way still similar to that of the lead, the hydrogen bond at the central anthranilic core is conserved, and differing only at the Leu side chain, which is turned towards the indole ring, and at the indole system, which is also somewhat turned towards the Leu chain. This leads to a loss of coplanarity between the indole and anthranilic systems, but allows to establish hydrophobic interactions between the indole ring and the Leu methyls. This tendency is enhanced in the Met derivative **5**: the conformation reported in Figure 1c is 4 kcal/mol lower in enthalpy of formation than that corresponding to **VL-0395**, and the geometry is completely different. The central hydrogen bond is lost, and replaced by a new bond between the anthranil amide hydrogen and the carboxylic end of the molecule. The amide groups of the molecule are thus pointing to opposite sides, and there is a strong interaction between the longer Met side chain and the indole ring. These 'closed' conformations are likely to be even more favoured in water, since they allow a minimization of the hydrophobic surface exposed to the solvent, and can be regarded as rather realistic. However, since the affinities of compounds **4** and **5** are similar, we have also considered the possibility that, within low energy conformations of the two compounds, similar families of conformations could be found. We have thus submitted the two compounds, and **VL-0395** as well, to a molecular dynamics run at 300 K, and we have performed a population analysis. Figure 3 reports the results of this analysis, taking as a conformation descriptor the distance between the carboxylic carbon and the anthranil amide hydrogen. It can be clearly seen that the minimum energy conformations are also the only ones populated at room temperature, at least within this model. Similar results are obtained considering other descriptors (torsion of Φ angle).

As a consequence, with this aliphatic-end compounds is difficult to find a relationship between the activity and the distance between the ends of the molecules in their ground state. If we consider the dependence of the activity upon the hydrophobic surfaces and volumes of the aminoacid side chains (**VL-0395**: area 98.4, volume 120.4; compound **4**: area 75.8, volume 81.6; compound **5**: area 86.3, volume 91.4), we see that the best compound in the series (**5**) bears a group of intermediate area and volume, and that perhaps the benzyl group of the lead is somewhat too large. On the other hand, the length of the aliphatic chains and their mobility could play a major role in improving the activity.

4.3. SAR of the free carboxy group

In addition to the above described derivatives, compounds **12–21** have been synthesized in order to elucidate the role of the aminoacidic free carboxy group in the receptor binding.

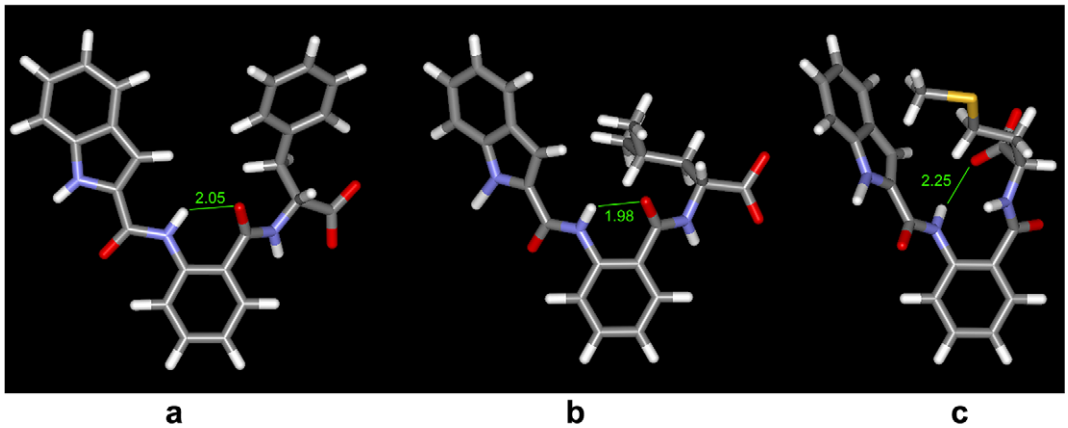


Figure 2. NMR-derived conformation of **VL-0395** (a) and lowest energy conformations of the higher affinity compounds **4** (b) and **5** (c).

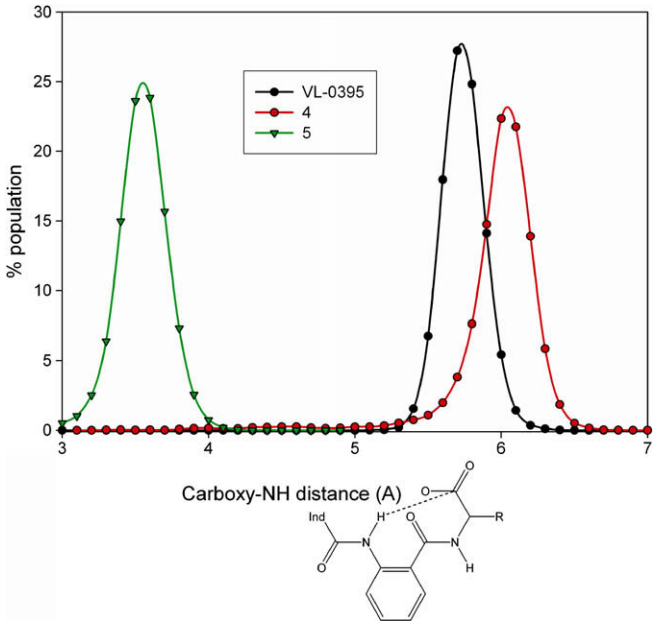


Figure 3. Population analysis of low energy conformations of **VL-0395** and of compounds **4** and **5** after molecular dynamics at 300 K.

The affinity data obtained for compounds **12–18** demonstrate clearly that the free carboxy group of the aminoacid is essential for the receptor binding. In fact, the remarkable drop in receptor affinity of the primary amide (**12**) in addition to the inactivity of the ethyl ester (**14**) confirms that the free carboxy group is engaged in the receptor binding through ionic or reinforced-ionic bond formation rather than in a double hydrogen bond interaction. Nevertheless, since the double hydrogen bond formation can be established even by the primary amide the 43-fold decrease of its affinity respect **VL-0395** exclude this type of interaction. Still the tertiary amide **13**, bearing the same alkyl substituents on the amido nitrogen of the known glutamic acid based CCK_1 receptor antagonist Lorglumide, was poorly tolerated in the receptor. Reduction of the carboxylic acid group to alcohol or to methyl group (**15** and **16**, respectively) confirms the loss of the affinity observed for the amide and ester derivatives. On the other hand, derivative **16**, although its reasonably no drug-like feature due to the presence of amphetamine, showed the better affinity among this subset of compounds. Deletion of the carboxylic acid functional group of **VL-0395** as well as that of compound **9** led to compounds **17** and **18**, respectively, lacking in affinity towards CCK_1 receptors. Their inactivity seems plausible due to both effects, that is, the deletion of the carboxylic group as well as to the abolishment of the asymmetry center in alpha position that ensured the correct spatial position of the chain toward the receptor binding pocket.

Having demonstrate that the carboxylic acid functional group is essential for the receptor binding we focused our attention on the correct position of this group in order to retain the receptor affinity. When the free carboxy group of the Gly derivative (**1**) was prolonged by one or two methylene groups the resulted compounds **19** and **20**, respectively, were inactive at the maximum dose tested of 3 μM. Moreover, in order to exclude that the loss in affinity observed of compounds **19** and **20** was due to the lack of the aminoacidic side chain we have also prolonged, by one more methylene group, the position of the carboxylic acid of the reference compound **VL-0395**. The failure in affinity, even at the forced maximum dose of 10 μM, of the β-Phe derivative (**21**) confirms that the correct position of the free carboxy group is the alpha position respect to the side chain. It is interesting to notice that the β-Phe residue confers the better affinity in the series of the peptoid derivatives like PD-140,548.[19]

All these findings suggests that in proximity of the hydrophobic pocket that accommodates the aminoacidic side chains of the above described anthranilic acid type ligands there is probably at least one aminoacid of the receptor that is implicated in ionic bond formation with the aminoacidic carboxy group.

4.4. Receptor stereoselectivity

Finally, compounds **22–25** were synthesized in order to validate the influence of the stereochemistry of the aminoacidic chiral center of the two more active compounds of this series (**4** and **5**) on the receptor affinity. Hence, we determined the eutomers and their selectivity indices (SI) as well as the eudismic ratio (ER) of the pure enantiomers of compounds **4** and **5**.

The binding data reported in Table 1 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 **23** and **25** are about 100-fold ($ER \approx 100$) more active than their distomers **22** and **24**, respectively.

Although the similar stereoselectivity ($ER \approx 100$) expressed by the two eutomers the highest selectivity versus CCK_1 receptors ($SI = 422$) was observed for the eutomer of the Met derivative (compound **25**).

5. Conclusion

In conclusion, by this SAR study dedicated to the C-terminal optimization of the anthranilic acid derivatives, we have demonstrate the following structural requirements:

- The receptor affinity depends on the nature of the aminoacidic side chain. Indeed, aminoacids of the endogenous ligand CCK-8, offers a different CCK_1 receptor 'address' type contribute and the order of the relative importance is as follow: Met > Phe > Trp > Tyr > Gly > Asp.
- The presence of an aromatic ring at the end of the aminoacidic side chain is not mandatory since linear or branched aliphatic chains produce a comparable or better affinity.
- Hydrophilic side chains are not tolerated in the receptor pocket confirming thus its hydrophobic nature.
- The aminoacidic free carboxy group is engaged in the receptor recognition through ionic bond formation and is essential for the binding.
- The correct position of the aminoacidic free carboxy group is the alpha-position respect to the side chain.
- The *R* configuration of the aminoacidic chiral center is preferred by the CCK1 receptor.

If all these findings are correct, an appropriate variation of the aminoacidic side chain could provide a better CCK_1 receptor affinity refinement.

6. Experimental

6.1. Chemical procedures

All chemicals and solvents used in syntheses were reagent-grade products and were used without additional purification. Reaction progress was monitored by ascending thin-layer chromatography (TLC) using precoated silica gel plates (60F-254 Merck). Visualization of the chromatograms was achieved by short wave UV light (254 nm). 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). 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). Optical rotations were determined with a Perkin–Elmer 241 polarimeter in a 1.0 dm tube. Determination of enantiomeric excesses was run on a Jasco HPLC chromatographer on CSP-TE-SP-100 (250 * 4 mm ID) at a flow rate of 1.00 mL/min of a mixture of ammonium acetate (20 mM) and methanol/water 85/15 (v/v), with an UV detection at 254 nm. Elemental analyses were performed by the Microanalyses Laboratory of the Department of Chemistry of the University of Trieste and were within ±0.4% of the theoretical values calculated for C, H and N.

6.2. General method for preparation of compound 1a–5a, 7a–9a, 14a, 19a–21a

Gaseous HCl was bubbled for 30 min. into a stirred suspension of 20.0 mmol of the aminoacid in 150 mL of abs. EtOH, cooled to 0 °C. The solvent was removed under vacuum and the residue was triturated with cold diethyl ether and filtered. The aminoacid ethyl esters hydrochlorides were obtained in almost quantitative yield and were used in the next step without further purification.

6.2.1. 2-(*R,S*)-Amino-3-(4-benzyloxy-phenyl)-propionic acid benzyl ester hydrochloride (11a)

Benzyl bromide (10.21 mL, 85.7 mmol) was added to a suspension of *N*-Boc-DL-tyrosine (6.00 g, 21.3 mmol) and K_2CO_3 (8.85 g, 64.0 mmol) in 100 mL of acetone and the reaction mixture was refluxed overnight under stirring. The solvent was removed under vacuum and the residue was partitioned between H_2O and diethyl ether. The aqueous phase was extracted three times with ether and the combined organic layers were washed with brine, dried over anhydrous sodium sulfate and evaporated to dryness. The residue was triturated with petroleum ether 40–60° and the precipitate formed was collected by filtration. *O*-Benzyl-*N*-Boc-DL-tyrosine benzyl ester (6.50 g, 66%) was obtained as a white powder. TLC (EtOAc/*n*-hexane 1:1). R_f 0.77; mp 88–89 °C; ^{1}H NMR ($CDCl_3$) δ 1.41 (t, 9H, -$(CH_3)_3$); 3.03 (m, 2H, –CH_2–CH<); 4.58 (m, 1H, –CH<); 5.02 (s, 2H, –CH_2–O–); 5.13 (s, 2H, –CH_2–O–); 6.63 (m, 2H, Ar); 6.94 (m, 2H, Ar); 7.34–7.41 (m, 10H, Ar); ^{13}C NMR ($CDCl_3$) δ 28.32, 37.38, 54.50, 67.07, 69.94, 79.89, 114.76, 127.42, 127.92, 128.40, 128.53, 130.30, 135.10, 136.88, 154.98, 157.74, 171.69.

O-Benzyl-*N*-Boc-dl-tyrosine benzyl ester (6.25 g, 13.5 mmol) was dissolved in 100 ml of ethyl acetate and gaseous HCl was bubbled at 0 °C for 5 min. After stirring at r.t. for 15 min, the solvent was removed under vacuum and the residue was triturated with cold diethyl ether and the precipitate formed was collected by filtration. *O*-Benzyl-dl-tyrosine benzyl ester hydrochloride (5.37 g, 99%) was obtained as a white solid. TLC (DCM/MeOH/H_2O/AcOH 9:1:0.1:0.1). R_f 0.51; mp 208–209 °C; ^{1}H NMR (DMSO-d_6) δ 3.07 (m, 2H, –CH_2–CH<); 4.23 (m, 1H, –CH<); 5.05 (s, 2H, –CH_2–O–); 5.11 (s, 2H, –CH_2–O–); 6.88 (m, 2H, Ar); 7.08 (m, 2H, Ar); 7.27–7.41 (m, 10H, Ar); 8.75 (b, 3H, NH_3^+); ^{13}C NMR (DMSO-d_6) δ 35.03, 53.27, 66.91, 69.08, 114.69, 126.42, 127.54, 127.73, 128.26, 128.33, 130.46, 134.73, 136.95, 157.43, 168.79.

6.3. General method for preparation of compound 1b–5b, 7b, 9b, 11b, 14b, 16b–25b

A suspension of 20.0 mmol of the aminoacid ethyl or benzyl ester hydrochloride (**1a**–**5a**, **7a**, **9a**, **11a**, **14a** and **19a–25a**) and TEA (2.78 mL, 20.0 mmol) or a solution of the amine (amphetamine, 2-phenylethylamine, tryptamine for compounds **16b**–**18b,** respectively) in 150 mL of ethyl acetate was treated with isatoic anhydride (3.26 g, 20.0 mmol). The resulting mixture was refluxed under stirring for 4 h, cooled to room temperature and filtered. The organic phase was washed with 1 N NaOH (3 × 50 mL), water (1 × 50 mL) and brine, dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was triturated with petroleum ether 40–60° and the precipitate was collected by filtration.

6.3.1. (2-Amino-benzoylamino)-acetic acid ethyl ester (1b)[23]

The titled compound was obtained in 66% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.52; mp 70–72 °C; ^{1}H NMR ($CDCl_3$) δ 1.26 (t, 3H, –CH_3); 4.18 (m, 4H, –CH_2–O– and –CH_2–CO–); 5.41 (b, 2H, –NH_2); 6.57–6.73 (m, 3H, –NH– and Ar); 7.17 (t, 1H, Ar); 7.38 (d, 1H, Ar); ^{13}C NMR ($CDCl_3$) δ 14.19, 41.62, 61.61, 115.18, 116.63, 117.30, 127.57, 132.61, 148.83, 169.35, 170.26.

6.3.2. 2-(*R,S*)-(2-Amino-benzoylamino)-propionic acid ethyl ester (2b)

The titled compound was obtained in 53% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.63; mp 95–97 °C; ^{1}H NMR ($CDCl_3$) δ 1.29 (t, 3H, –CH_2–CH_3); 1.48 (d, 3H, >CH–CH_3); 4.22 (q, 2H, –CH_2–); 4.71 (m, 1H, –CH<); 5.15 (b, 2H, –NH_2); 6.64 (m, 3H, –NH– and Ar); 7.19 (t, 1H, Ar); 7.38 (d, 1H, Ar); ^{13}C NMR ($CDCl_3$) δ 14.19, 18.66, 48.30, 61.59, 115.42, 116.63, 117.29, 127.47, 132.54, 148.84, 168.70, 173.32.

6.3.3. 2-(*R,S*)-(2-Amino-benzoylamino)-3-methyl-butyric acid ethyl ester (3b)[23]

Crystallization from EtOAc/*n*-hexane afforded the titled compound in 65% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.70; mp 48 °C; ^{1}H NMR ($CDCl_3$) δ 0.97 (m, 6H, –CH(CH_3)$_2$); 1.28 (t, 3H, –CH_2–CH_3); 2.24 (m, 1H, –CH(CH_3)$_2$); 4.20 (m, 2H, –CH_2–O–); 4.68 (m, 1H, –NH–CH<); 5.46 (b, 2H, –NH_2); 6.56 (d, 1H, –NH–); 6.64 (m, 2H, Ar); 7.19 (t, 1H, Ar); 7.39 (d, 1H, Ar); ^{13}C NMR ($CDCl_3$) δ 14.40, 18.10, 19.17, 31.70, 57.14, 61.46, 115.78, 116.70, 117.32, 127.46, 132.56, 148.77, 169.02, 172.23.

6.3.4. 2-(*R,S*)-(2-Amino-benzoylamino)-4-methyl-pentanoic acid ethyl ester (4b)[23]

The titled compound was obtained in 75% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.73; mp 49–50 °C; ^{1}H NMR ($CDCl_3$) δ 0.97 (m, 6H, –CH(CH_3)$_2$); 1.27 (t, 3H, –CH_2–CH_3); 1.69 (m, 3H, –CH_2–CH< and –CH(CH_3)$_2$); 4.19 (q, 2H, –CH_2–O–); 4.77 (m, 3H, –NH–CH< and –NH_2); 6.52 (d, 1H, –NH–); 6.59–6.66 (m, 2H, Ar); 7.18 (t, 1H, Ar); 7.38 (d, 1H, Ar); ^{13}C NMR ($CDCl_3$) δ 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, 168.94, 173.34.

6.3.5. 2-(*R,S*)-(2-Amino-benzoylamino)-4-methylsulfanyl-butyric acid ethyl ester (5b)[23]

The titled compound was obtained in 70% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.63; mp 53–55 °C; ^{1}H NMR ($CDCl_3$) δ 1.30 (t, 3H, –CH_2–CH_3); 2.10 (s, 3H, –S–CH_3); 2.22 (m, 2H, –CH_2–CH<); 2.57 (m, 2H, –CH_2–S–); 4.23 (q, 2H, –CH_2–O–); 4.84 (m, 3H, –CH< and –NH_2); 6.64 (m, 2H, Ar); 6.82 (d, 1H, –NH–); 7.20 (t, 1H, Ar); 7.40 (d, 1H, Ar); ^{13}C NMR ($CDCl_3$) δ 14.22, 15.58, 30.15, 31.86, 51.82, 61.77, 115.16, 116.65, 117.34, 127.46, 132.67, 148.94, 169.00, 172.20.

6.3.6. 2-(*R,S*)-(2-Amino-benzoylamino)-succinic acid diethyl ester (7b)[23]

Purification by flash chromatography (CH_2Cl_2 to EtOAc/CH_2Cl_2 1:4) afforded the titled compound in 59% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.56; mp 89–90 °C; ^{1}H NMR ($CDCl_3$) δ 1.27 (m, 6H, $-CH_3$); 3.02 (m, 2H, $-CH_2-CH<$); 4.16 (q, 2H, $-CH_2-O-$); 4.25 (q, 2H, $-CH_2-O-$); 5.00 (m, 1H, $-CH<$); 5.52 (b, 2H, $-NH_2$); 6.67 (m, 2H, Ar); 7.15 (d, 1H, –NH–); 7.23 (t, 1H, Ar); 7.41 (d, 1H, Ar); ^{13}C NMR ($CDCl_3$) δ 14.27, 36.57, 48.77, 61.20, 62.07, 115.16, 116.78, 117.32, 127.68, 132.74, 148.86, 168.69, 170.98, 171.20.

6.3.7. 2-(*R,S*)-(2-Amino-benzoylamino)-3-(1*H*-indol-3-yl)-propionic acid ethyl ester (9b)[23]

The titled compound was obtained in 81% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.49; mp 98–100 °C; ^{1}H NMR ($CDCl_3$) δ 1.24 (t, 3H, $-CH_3$); 3.42 (m, 2H, $-CH_2-CH<$); 4.16 (q, 2H, $-CH_2-O-$); 5.06 (m, 1H, $-CH<$); 5.45 (b, 2H, $-NH_2$); 6.52–6.67 (m, 3H, Ar and –NH–); 7.02–7.26 (m, 5H, Ar); 7.35 (d, 1H, Ar); 7.58 (d, 1H, Ar); 8.13 (b, 1H, –NH–); ^{13}C NMR ($CDCl_3$) δ 14.14, 27.71, 53.27, 61.61, 110.08, 111.32, 115.49, 116.67, 117.28, 118.69, 119.66, 122.24, 122.92, 127.60, 127.66, 132.53, 136.09, 148.79, 168.65, 172.17.

6.3.8. 2-(*R,S*)-(2-Amino-benzoylamino)-3-(4-benzyloxy-phenyl)-propionic acid benzyl ester (11b)

Purification by flash chromatography (CH_2Cl_2 to 10% EtOAc/CH_2Cl_2) followed by trituration with cold petroleum ether 40–60° afforded the titled compound in 28% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.62; mp 109–110 °C; ^{1}H NMR ($CDCl_3$) δ 3.19 (m, 2H, $-CH_2-CH<$); 5.08 (m, 3H, $-CH_2-O-$ and $-CH<$); 5.21 (m, 2H, $-CH_2-O-$); 5.49 (b, 2H,—NH_2); 6.53 (d, 1H, –NH–); 6.61–6.71 (m, 2H, Ar); 6.85 (d, 2H, Ar); 6.96 (d, 2H, Ar); 7.20–7.44 (m, 12H, Ar); ^{13}C NMR ($CDCl_3$) δ 37.02, 53.37, 67.29, 69.94, 114.85, 115.23, 116.60, 117.19, 127.33, 127.42, 127.86, 127.90, 128.50, 128.56, 128.62, 130.34, 132.51, 135.01, 136.85, 148.71, 157.79, 168.49, 171.50.

6.3.9. 2-(*R,S*)-(2-Amino-benzoylamino)-3-phenyl-propionic acid ethyl ester (14b)

Obtained as previously described.[26]

6.3.10. (1*R,S*)-2-Amino-*N*-(1-methyl-2-phenyl-ethyl)-benzamide (16b)

Crystallization from EtOAc/*n*-hexane afforded the titled compound in 78% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.61; mp 103 °C; ^{1}H NMR ($CDCl_3$) δ 1.18 (d, 3H, $-CH_3$); 2.86 (m, 2H, $-CH_2-$); 4.41 (m, 1H, $-CH<$), 5.42 (b, 2H, $-NH_2$); 5.94 (d, 1H, –NH–); 6.56–6.65 (m, 2H, Ar); 7.13–7.33 (m, 7H, Ar); ^{13}C NMR ($CDCl_3$) δ 20.17, 42.54, 46.24, 116.58, 116.67, 117.31, 126.57, 127.10, 128.50, 129.60, 132.18, 137.98, 148.58, 168.64.

6.3.11. 2-Amino-*N*-phenethyl-benzamide (17b)[26]

The titled compound was obtained as an oil in 82% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.65; ^{1}H NMR ($CDCl_3$) δ 2.91 (t, 2H, $-CH_2-C_6H_5$); 3.65 (m, 2H, $-CH_2-NH-$); 5.40 (b, 2H, $-NH_2$); 6.19 (b, 1H, –NH–); 6.55–6.67 (m, 2H, Ar); 7.15–7.37 (m, 7H, Ar); ^{13}C NMR ($CDCl_3$) δ 35.79, 40.88, 116.17, 116.63, 117.30, 126.59, 127.11, 128.73, 128.85, 132.23, 139.04, 148.66, 169.34.

6.3.12. 2-Amino-*N*-[2-(1H-indol-3-yl)-ethyl]-benzamide (18b)[23]

Trituration with hot 95% EtOH afforded the titled compound in 83% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.43; mp 159–161 °C; ^{1}H NMR (DMSO-d_6) δ 2.96 (t, 2H, $-CH_2-CH_2-NH-$); 3.53 (m, 2H, $-CH_2-NH-$); 6.45 (b, 2H, $-NH_2$); 6.52 (t, 1H, Ar); 6.72 (d, 1H, Ar); 6.97–7.21 (m, 4H, Ar); 7.37 (d, 1H, Ar); 7.49 (d, 1H, Ar); 7.62 (d, 1H, Ar); 8.37 (t, 1H, –NH–); 10.84 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ 25.05, 39.64, 111.19, 111.81, 114.36, 114.79, 116.14, 118.04, 118.14, 120.74, 122.38, 127.11, 127.82, 131.34, 136.05, 149.40, 168.65.

6.3.13. 3-(2-Amino-benzoylamino)-propionic acid ethyl ester (19b)

Purification by flash chromatography (CH_2Cl_2 to EtOAc/CH_2Cl_2 2:3) afforded the titled compound in 70% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.35; mp 73 °C; ^{1}H NMR ($CDCl_3$) δ 1.27 (t, 3H, $-CH_3$); 2.62 (t, 2H, $-NH-CH_2-CH_2-$); 3.67 (m, 2H, $-NH-CH_2-CH_2-$); 4.16 (q, 2H, $-O-CH_2-$); 5.54 (b, 2H, $-NH_2$); 6.60–6.69 (m, 2H, Ar); 6.77 (m, 1H, –NH–); 7.15–7.32 (m, 2H, Ar); ^{13}C NMR ($CDCl_3$) δ 14.34, 34.16, 35.05, 60.92, 115.84, 116.66, 117.31, 127.27, 132.37, 148.79, 169.18, 172.87.

6.3.14. 4-(2-Amino-benzoylamino)-butyric acid ethyl ester (20b)

Purification by flash chromatography (CH_2Cl_2 to EtOAc/CH_2Cl_2 2:3) afforded the titled compound in 58% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.37; mp 39–41 °C; ^{1}H NMR ($CDCl_3$) δ 1.25 (t, 3H, $-CH_3$); 1.95 (m, 2H, $-NH-CH_2-CH_2-CH_2-$); 2.43 (t, 2H, $-NH-CH_2-CH_2-CH_2-$); 3.46 (m, 2H, $-NH-CH_2-CH_2-CH_2-$); 4.13 (q, 2H, $-O-CH_2-$); 5.55 (b, 2H, $-NH_2$); 6.42 (m, 1H, –NH–); 6.61–6.69 (m, 2H, Ar); 7.16–7.34 (m, 2H, Ar); ^{13}C NMR ($CDCl_3$) δ 14.35, 24.60, 32.11, 39.40, 60.78, 115.94, 116.63, 117.35, 127.11, 132.29, 148.78, 169.41, 173.73.

6.3.15. 3-(*R,S*)-(2-Amino-benzoylamino)-4-phenyl-butyric acid ethyl ester (21b)

Purification by flash chromatography (CH_2Cl_2 to EtOAc/CH_2Cl_2 1:4) afforded the titled compound in 48% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.50; mp 90 °C; ^{1}H NMR ($CDCl_3$) δ 1.28 (t, 3H, $-CH_3$); 2.57 (m, 2H, $-CH_2-CO-$); 2.91 (dd, 1H, $-CH_2-C_6H_5$); 3.07 (dd, 1H, $-CH_2-C_6H_5$); 4.17 (q, 2H, $-CH_2-O-$); 4.65 (m, 1H, $-CH<$); 5.49 (b, 2H, $-NH_2$); 6.64 (m, 2H, Ar); 6.87 (d, 1H, –*N*H); 7.15–7.35 (m, 7H, Ar); ^{13}C NMR ($CDCl_3$) δ 14.25, 37.02, 40.06, 47.37, 60.79, 115.97, 116.65, 117.21, 126.67, 127.12, 128.56, 129.29, 132.22, 137.55, 148.63, 168.40, 171.97.

6.3.16. 2(*S*)-(2-Amino-benzoylamino)-4-methyl-pentanoic acid ethyl ester (22b)

The titled compound was obtained in 70% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.73; $[\alpha]_D^{25}$ = +17.3 (*c* 1.2, CH_2Cl_2); mp 53–54 °C; ^{1}H NMR ($CDCl_3$) δ 0.97 (m, 6H, $-CH(CH_3)_2$); 1.25 (t, 3H, $-CH_2-CH_3$); 1.66 (m, 3H, $-CH_2-CH<$ and $-CH(CH_3)_2$); 4.21 (q, 2H, $-CH_2-O-$); 4.73 (m, 3H, $-NH-CH<$ and $-NH_2$); 6.50 (d, 1H, –NH–); 6.56–6.64 (m, 2H, Ar); 7.20 (t, 1H, Ar); 7.35 (d, 1H, Ar); ^{13}C NMR^{13}C NMR ($CDCl_3$) δ 14.20, 22.13, 22.85, 25.05, 41.80, 50.90, 61.39, 115.80, 116.60, 117.30, 127.40, 132.50, 148.80, 168.90, 173.30.

6.3.17. 2(*R*)-(2-Amino-benzoylamino)-4-methyl-pentanoic acid ethyl ester (23b)

The titled compound was obtained in 73% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.73; $[\alpha]_D^{25}$ = -16.1 (*c* 0.4, CH_2Cl_2); mp 50–51 °C; ^{1}H NMR ($CDCl_3$) δ 0.97 (m, 6H, $-CH(CH_3)_2$); 1.26 (t, 3H, $-CH_2-CH_3$); 1.65 (m, 3H, $-CH_2-CH<$ and $-CH(CH_3)_2$); 4.18 (q, 2H, $-CH_2-O-$); 4.74 (m, 3H, $-NH-CH<$ and $-NH_2$); 6.49 (d, 1H, –NH–); 6.55–6.65 (m, 2H, Ar); 7.17 (t, 1H, Ar); 7.36 (d, 1H, Ar); ^{13}C NMR ($CDCl_3$) δ 14.20, 22.14, 22.86, 25.03, 41.83, 50.92, 61.40, 115.82, 116.62, 117.31, 127.42, 132.54, 148.77, 168.92, 173.34.

6.3.18. 2(*S*)-(2-Amino-benzoylamino)-4-methylsulfanyl-butyric acid ethyl ester (24b)

The titled compound was obtained in 66% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.63; $[\alpha]_D^{25}$ = +35.3 (*c* 1.6, CH_2Cl_2); mp 55–57 °C; ^{1}H NMR ($CDCl_3$) δ 1.30 (t, 3H, $-CH_2-CH_3$); 2.13 (s, 3H, $-S-CH_3$); 2.25 (m, 2H, $-CH_2-CH<$); 2.60 (m, 2H, $-CH_2-S-$); 4.25 (q, 2H,

–CH_2–O–); 4.80 (m, 3H, –CH< and –NH_2); 6.60 (m, 2H, Ar); 6.79 (d, 1H, –NH–); 7.22 (t, 1H, Ar); 7.44 (d, 1H, Ar); ^{13}C NMR ($CDCl_3$) δ 14.20, 15.60, 30.18, 31.82, 51.88, 61.75, 115.10, 116.69, 117.30, 127.48, 132.70, 148.90, 169.20, 172.20.

6.3.19. 2-(*R*)-(2-Amino-benzoylamino)-4-methylsulfanyl-butyric acid ethyl ester (25b)

The titled compound was obtained in 72% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.63; $[\alpha]_D^{25}$ = -33.7 (*c* 1.2, CH_2Cl_2); mp 51–53 °C; ^{1}H NMR ($CDCl_3$) δ 1.28 (t, 3H, –CH_2–CH_3); 2.09 (s, 3H, –S–CH_3); 2.19 (m, 2H, –CH_2–CH<); 2.52 (m, 2H, –CH_2–S–); 4.20 (q, 2H, –CH_2–O–); 4.80 (m, 3H, –CH< and –NH_2); 6.65 (m, 2H, Ar); 6.80 (d, 1H, –NH–); 7.24 (t, 1H, Ar); 7.46 (d, 1H, Ar); ^{13}C NMR ($CDCl_3$) δ 14.23, 15.57, 30.14, 31.86, 51.85, 61.73, 115.15, 116.64, 117.35, 127.45, 132.65, 148.92, 169.00, 172.20.

6.3.20. 2-(*R,S*)-(2-Amino-benzoylamino)-pentanedioic acid diethyl ester (8b)

A solution of 4.80 g (20.0 mmol) of DL-glutamic acid diethyl ester hydrochloride **8a** and 2.78 mL (20.0 mmol) of TEA in 100 mL of CH_2Cl_2 was cooled to 0 °C, with stirring. *o*-Nitrobenzoyl chloride (3.71 g, 20.0 mmol) was added and the mixture was stirred for 2 h at rt. The organic phase was washed with 0.1 N NaOH, H_2O, brine, dried over anhydrous Na_2SO_4 and evaporated. The crude product was dissolved in 100 mL of CH_2Cl_2 and 10 g of Zn were added. The suspension was cooled to 0 °C and 12 mL of acetic acid were added dropwise under stirring. The reaction mixture was stirred for 1 h and filtered. The organic phase was evaporated and the residue was triturated with cold petroleum ether 40–60° to give 3.51 g (54%) of the titled compound. TLC (EtOAc/*n*-hexane 1:1). R_f 0.57; mp 78–80 °C; ^{1}H NMR ($CDCl_3$) δ 1.22 (t, 3H, –CH_3); 1.29 (t, 3H, –CH_3); 2.08–2.50 (m, 2H, –CH_2–CH<); 2.46 (m, 2H, –CH_2–CO–); 4.10 (q, 2H, –CH_2–O–); 4.24 (q, 2H, –CH_2–O–); 4.74 (m, 1H, –CH<); 5.55 (b, 2H, –NH_2); 6.65 (m, 2H, Ar); 6.88 (d, 1H, –NH–); 7.20 (t, 1H, Ar); 7.40 (d, 1H, Ar); ^{13}C NMR ($CDCl_3$) δ 14.20, 27.32, 30.53, 52.05, 60.83, 61.72, 114.93, 116.58, 117.31, 127.49, 132.66, 149.03, 169.04, 172.12, 173.18.

6.4. General procedure for the synthesis of derivatives 1c–9c, 11c, 14, 16–18, 19c–25c

To a suspension of 10.0 mmol of indole-2-carboxylic acid (for synthesis of compound **1c**–**5c**, **7c**–**9c**, **11c**, **14**, **16**–**18**, **19c**–**25c**) or *N*-methylindole-2-carboxylic acid (for synthesis of compound **6c**) in 30 mL of acetyl chloride cooled in an ice-bath were added portionwise, over a period of 0.5 h, 2.08 g (10.0 mmol) of PCl_5. After the mixture turned into a clear solution, stirring was continued at room temperature for 3 h. 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 7.50 mmol of the derivatives **1b**–**5b**, **7b**–**9b**, **14b**, **16b**–**25b** in 10 mL of pyridine. After the addition was complete, the reaction mixture was stirred at room temperature until completion (TLC monitoring). Compounds **1c**, **2c** and **5c** precipitated from the reaction mixture and were filtered. For the others compound, CH_2Cl_2 (150 mL) was added and the organic layer was washed twice with 40 mL of 1 N HCl, H_2O, 1 N NaOH and brine. After drying over anhydrous Na_2SO_4, the organic phase was rotary evaporated and the residue was purified as described to yield the titled compounds.

6.4.1. {2-[(1*H*-Indole-2-carbonyl)-amino]-benzoylamino}-acetic acid ethyl ester (1c)

Trituration with cold petroleum ether 40–60° afforded the titled compound in 76% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.52; mp 244–246 °C; ^{1}H NMR (DMSO-d_6) δ 1.22 (t, 3H, –CH_3); 4.12 (m, 4H, –CH_2–CO– and –CH_2–O–); 7.07 (m, 2H, Ar); 7.24 (m, 2H, Ar); 7.49 (d, 1H, Ar); 7.63 (t, 1H, Ar); 7.71 (d, 1H, Ar); 7.90 (d, 1H, Ar); 8.69 (d, 1H, Ar); 9.39 (t, 1H, –N*H*–CH_2–); 11.94 (s, 1H, –NH–); 12.48 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ 13.91, 41.26, 60.49, 102.45, 112.33, 118.76, 119.83, 120.03, 121.62, 122.48, 123.91, 126.79, 128.09, 131.42, 132.59, 136.95, 139.31, 159.01, 169.06, 169.26.

6.4.2. 2-(*R,S*)-{2-[(1*H*-Indole-2-carbonyl)-amino]-benzoyl-amino}-propionic acid ethyl ester (2c)

Trituration with $CHCl_3$ afforded the titled compound in 49% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.57; mp 262–264 °C; ^{1}H NMR (DMSO-d_6) δ 1.20 (t, 3H, –CH_2–CH_3); 1.45 (d, 3H, >CH–CH_3); 4.14 (q, 2H, –CH_2–); 4.58 (m, 1H, –CH<); 7.09 (m, 2H, Ar); 7.24 (m, 2H, Ar); 7.49 (d, 1H, Ar); 7.62 (t, 1H, Ar); 7.71 (d, 1H, Ar); 7.93 (d, 1H, Ar); 8.66 (d, 1H, Ar); 9.23 (d, 1H, –N*H*–CH<); 11.95 (s, 1H, –NH–); 12.32 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ 13.88, 16.24, 48.42, 60.45, 102.41, 112.33, 119.23, 119.81, 120.03, 121.58, 122.38, 123.89, 126.78, 128.48, 131.42, 132.41, 136.96, 139.06, 158.99, 168.65, 172.08.

6.4.3. 2-(*R,S*)-{2-[(1*H*-Indole-2-carbonyl)-amino]-benzoyl-amino}-3-methyl-butyric acid ethyl ester (3c)

Trituration with hot MeOH followed by flash chromatography (CH_2Cl_2 to EtOAc/CH_2Cl_2 1:4) afforded the titled compound in 54% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.71; mp 211 °C; ^{1}H NMR (DMSO-d_6) δ 0.96 (m, 6H, –CH(CH_3)$_2$); 1.17 (t, 3H, –CH_2–CH_3); 2.20 (m, 1H, –CH(CH_3)$_2$); 4.11 (q, 2H, –CH_2–); 4.31 (m, 1H, –NH–CH<); 7.00 (s, 1H, Ar); 7.07 (d, 1H, Ar); 7.20 (m, 2H, Ar); 7.44 (d, 1H, Ar); 7.58 (t, 1H, Ar); 7.66 (d, 1H, Ar); 7.89 (d, 1H, Ar); 8.57 (d, 1H, Ar); 9.01 (d, 1H, –N*H*–CH<); 11.90 (s, 1H, –NH–); 12.06 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ 14.94, 19.89, 30.10, 59.67, 61.22, 103.23, 113.23, 120.63, 120.74, 120.94, 122.47, 123.35, 124.82, 127.63, 129.79, 132.22, 133.25, 137.80, 139.57, 159.79, 170.01, 171.97.

6.4.4. 2-(*R,S*)-{2-[(1*H*-Indole-2-carbonyl)-amino]-benzoyl-amino}-4-methyl-pentanoic acid ethyl ester (4c)

Trituration with hot MeOH afforded the titled compound in 67% yield. TLC (EtOAc/*n*-hexane 1:1). R_f. R_f 0.73; mp 202–203 °C; ^{1}H NMR ($CDCl_3$) δ 0.96 (dd, 6H, –CH(CH_3)$_2$); 1.34 (t, 3H, –CH_2–CH_3); 1.79 (m, 3H, –CH_2–CH(CH_3)$_2$); 4.28 (q, 2H, –CH_2–O–); 4.94 (m, 1H, –NH–CH<); 7.05–7.31 (m, 5H, Ar and –N*H*–CH<); 7.50 (m, 2H, Ar); 7.68 (m, 2H, Ar); 8.80 (d, 1H, Ar); 10.00 (s, 1H, –NH–); 12.22 (s, 1H, –NH–); ^{13}C NMR ($CDCl_3$) δ 14.26, 22.09, 22.97, 25.14, 41.59, 51.34, 61.80, 103.93, 112.11, 119.48, 120.61, 121.21, 122.33, 122.84, 124.69, 126.99, 127.86, 131.45, 133.04, 136.94, 139.90, 160.16, 168.99, 173.24.

6.4.5. 2-(*R,S*)-{2-[(1*H*-Indole-2-carbonyl)-amino]-benzoylamino}-4-methylsulfanyl-butyric acid ethyl ester (5c)

Crystallization with MeOH afforded the titled compound in 85% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.49; mp 216–218 °C; ^{1}H NMR (DMSO-d_6) δ 1.32 (t, 3H, –CH_2–CH_3); 2.07 (s, 3H, CH_3–S–); 2.14 (m, 2H, –CH_2–CH<); 2.62 (m, 2H, –CH_2–S–); 4.15 (q, 2H, –CH_2–O–); 4.67 (m, 1H, –NH–CH<); 7.09 (m, 2H, Ar); 7.25 (m, 2H, Ar); 7.49 (d, 1H, Ar); 7.59–7.73 (m, 2H, Ar); 7.94 (d, 1H, Ar); 8.65 (d, 1H, Ar); 9.21 (d, 1H, –N*H*–CH<); 11.95 (s, 1H, –NH–); 12.24 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ 13.89, 14.36, 29.62, 29.73, 51.76, 60.62, 102.40, 112.33, 119.34, 119.83, 120.04, 121.59, 122.44, 123.91, 126.77, 128.57, 131.38, 132.48, 136.95, 138.96, 158.97, 169.13, 171.35.

6.4.6. 2-(*R,S*)-{2-[(1-Methyl-1*H*-indole-2-carbonyl)-amino]-benzoylamino}-4-methylsulfanyl-butyric acid ethyl ester (6c)

The titled compound wasn't isolated but used in the next step without purification and characterization.

6.4.7. 2-(*R,S*)-{2-[(1*H*-Indole-2-carbonyl)-amino]-benzoylamino}-succinic acid diethyl ester (7c)

Trituration with hot MeOH afforded the titled compound in 90% yield. TLC (EtOAc/*n*-hexane 1:1) R_f 0.56; mp 199–200 °C; ^{1}H NMR (DMSO-d_6) δ 1.19 (m, 6H, –CH_3); 2.93 (m, 2H, –CH_2–CH<); 4.10 (m, 4H, –CH_2–O–); 4.92 (m, 1H, –CH<); 7.06 (s, 1H, Ar); 7.11 (d, 1H, Ar); 7.25 (m, 2H, Ar); 7.48 (d, 1H, Ar); 7.63 (t, 1H, Ar); 7.73 (d, 1H, Ar); 7.84 (d, 1H, Ar); 8.64 (d, 1H, Ar); 9.33 (d, 1H, –N*H*–CH<); 11.94 (s, 1H, –NH–); 12.24 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ 14.76, 14.81, 36.12, 50.12, 61.14, 61.88, 103.33, 113.22, 119.98, 120.83, 120.95, 122.56, 123.41, 124.83, 127.66, 129.19, 132.20, 133.52, 137.83, 139.92, 159.85, 169.39, 170.62, 170.98.

6.4.8. 2-(*R,S*)-{2-[(1*H*-Indole-2-carbonyl)-amino]-benzoylamino}-pentanedioic acid diethyl ester (8c)

Trituration with hot MeOH afforded the titled compound in 86% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.56; mp 195–197 °C; ^{1}H NMR ($CDCl_3$) δ 1.21 (t, 3H, –CH_3); 1.32 (t, 3H, –CH_3); 2.19–2.60 (m, 2H, –CH_2–CH<); 2.54 (m, 2H, –CH_2–CO-); 4.12 (q, 2H, –CH_2–O–); 4.27 (q, 2H, –CH_2–O–); 4.86 (m, 1H, –CH<); 7.07–7.32 (m, 4H, Ar); 7.49 (m, 3H, Ar and –N*H*–CH<); 7.69 (m, 2H, Ar); 8.83 (d, 1H, Ar); 9.86 (s, 1H, –NH–); 12.36 (s, 1H, –NH–); ^{13}C NMR ($CDCl_3$) δ 14.17, 14.21, 26.94, 30.51, 52.62, 61.06, 62.02, 103.90, 112.04, 118.89, 120.62, 121.19, 122.35, 122.84, 124.69, 127.07, 127.88, 131.50, 133.21, 136.87, 140.18, 160.11, 169.09, 171.77, 173.39.

6.4.9. 2-(*R,S*)-{2-[(1*H*-Indole-2-carbonyl)-amino]-benzoylamino}-3-(1*H*-indol-3-yl)-propionic acid ethyl ester (9c)

Crystallization with MeOH afforded the titled compound in 85% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.49; mp 216–218 °C; ^{1}H NMR (DMSO-d_6) δ 1.16 (t, 3H, –CH_3); 3.37 (m, 2H, –CH_2–CH<); 4.13 (q, 2H, –CH_2–O–); 4.78 (m, 1H, –CH<); 6.98–7.35 (m, 8H, Ar); 7.46–7.69 (m, 4H, Ar); 7.84 (d, 1H, Ar); 8.67 (d, 1H, Ar); 9.23 (d, 1H, –N*H*–CH<); 10.85 (s, 1H, –NH–); 11.89 (s, 1H, –NH–); 12.28 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ 13.81, 26.25, 53.86, 60.47, 102.33, 109.60, 111.26, 112.28, 117.78, 118.20, 118.95, 119.66, 119.81, 120.78, 121.52, 122.14, 123.45, 123.75, 126.77, 126.85, 128.39, 131.40, 132.33, 135.96, 136.93, 139.19, 158.94, 168.79, 171.37.

6.4.10. (2*R,S*)-3-(4-Benzyloxy-phenyl)-2-{2-[(1*H*-indole-2-carbonyl)-amino]-benzoylamino}-propionic acid benzyl ester (11c)

Trituration with MeOH, with cold diethyl ether followed by flash chromatography (petroleum ether 40–60°/CH_2Cl_2 1:1 to CH_2Cl_2 to EtOAc/CH_2Cl_2 1:9) afforded the titled compound in 26% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.52; mp 179–180 °C; ^{1}H NMR (DMSO-d_6) δ 3.15 (m, 2H, –CH_2–CH<); 4.84 (m, 1H, –CH<); 4.94 (s, 2H, –CH_2–O–); 5.16 (s, 2H, –CH_2–O–); 6.87–6.98 (m, 3H, Ar); 7.06–7.13 (m, 2H, Ar); 7.21–7.33 (m, 13H, Ar); 7.48 (d, 1H, Ar); 7.61 (t, 1H, Ar); 7.70 (d, 1H, Ar); 7.80 (d, 1H, Ar); 8.64 (d, 1H, Ar); 9.32 (d, 1H, –N*H*–CH<); 11.95 (s, 1H, –NH–); 12.21 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ 35.16, 54.51, 66.08, 68.94, 102.47, 112.41, 114.39, 118.97, 119.77, 120.11, 121.73, 122.46, 124.01, 126.84, 127.53, 127.61, 127.87, 128.21, 128.37, 129.29, 130.05, 131.39, 132.64, 135.69, 136.90, 137.01, 139.11, 156.94, 158.96, 168.80, 171.03.

6.4.11. 2-(*R,S*)-{2-[(1*H*-Indole-2-carbonyl)-amino]-benzoylamino}-3-phenyl-propionic acid ethyl ester (14)

Obtained as previously described.[26]

6.4.12. (1*R,S*)-1*H*-Indole-2-carboxylic acid [2-(1-methyl-2-phenyl-ethylcarbamoyl)-phenyl]-amide (16)

Trituration with hot MeOH followed by flash chromatography (CH_2Cl_2 to EtOAc/CH_2Cl_2 1:4) afforded the titled compound in 64% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.73; mp 248–249 °C; ^{1}H NMR^{1}H NMR (DMSO-d_6) δ 1.23 (d, 3H, –CH_3); 2.87 (m, 2H, –CH_2–); 4.40 (m, 1H, –CH<); 7.03 (s, 1H, Ar); 7.07–7.28 (m, 8H, ar); 7.54 (m, 2H, Ar); 7.78 (m, 2H, Ar); 8.63 (d, 1H, Ar); 8.76 (d, 1H, –N*H*–CH<); 11.93 (s, 1H, –NH–); 12.48 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ 21.06, 42.38, 47.49, 103.33, 113.20, 120.57, 120.76, 120.88, 122.56, 123.16, 124.73, 126.76, 127.69, 128.78, 128.91, 129.81, 132.36, 132.86, 137.78, 139.75, 139.87, 159.79, 168.44. MS (ES) *m/z* 398.1 [MH]$^+$; MW: 397.48 (calcd. for $C_{25}H_{23}N_3O_2$).

6.4.13. 1*H*-Indole-2-carboxylic acid (2-phenethylcarbamoyl-phenyl)-amide (17)

Trituration with hot $CHCl_3$ afforded the titled compound in 65% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.63; mp 257–259 °C; ^{1}H NMR (DMSO-d_6) δ 2.92 (t, 2H, –CH_2–C_6H_5); 3.58 (m, 2H, –CH_2–NH–); 7.07 (s, 1H, Ar); 7.11–7.30 (m, 8H, Ar); 7.54 (m, 2H, Ar); 7.79 (m, 2H, Ar); 8.68 (d, 1H, Ar); 9.02 (t, 1H, –N*H*–CH_2-); 11.95 (s, 1H, –NH–); 12.64 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ 34.60, 40.59, 102.48, 112.32, 119.59, 119.76, 120.00, 121.68, 122.35, 123.86, 125.95, 126.86, 127.95, 128.11, 128.51, 131.52, 132.09, 136.94, 139.18, 159.00, 168.37. MS (ES) *m/z* 384.2 [MH]$^+$; MW: 383.45 (calcd for $C_{24}H_{21}N_3O_2$).

6.4.14. 1*H*-Indole-2-carboxylic acid {2-[2-(1*H*-indol-3-yl)-ethylcarbamoyl]-phenyl}-amide (18)

Trituration with EtOH and with hot isopropyl acetate afforded the titled compound in 49% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.46; mp 254–256 °C; ^{1}H NMR (DMSO-d_6) δ 3.05 (t, 2H, –CH_2–CH_2–NH–); 3.65 (m, 2H, –CH_2–NH–); 7.00–7.38 (m, 8H, Ar); 7.49–7.65 (m, 3H, Ar); 7.75 (d, 1H, Ar); 7.85 (d, 1H, Ar); 8.70 (d, 1H, Ar); 9.08 (t, 1H, –CH_2–N*H*-); 10.87 (s, 1H, –NH–); 11.95 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ 24.62, 40.13, 102.40, 111.22, 111.52, 112.32, 118.06, 119.55, 119.74, 120.00, 120.77, 121.68, 122.31, 122.52, 123.85, 126.86, 127.09, 128.02, 131.59, 132.09, 136.06, 136.93, 139.28, 159.01, 168.40. MS (ES) *m/z* 423.1 [MH]$^+$; MW: 422.49 (calcd for $C_{26}H_{22}N_4O_2$).

6.4.15. 3-{2-[(1*H*-Indole-2-carbonyl)-amino]-benzoylamino}-propionic acid ethyl ester (19c)

Trituration with hot MeOH followed by flash chromatography (CH_2Cl_2 to EtOAc/CH_2Cl_2 1:4) afforded the titled compound in 67% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.51; mp 204 °C; ^{1}H NMR (DMSO-d_6) δ1.13 (t, 3H, –CH_3); 2.61 (t, 2H, –NH–CH_2–CH_2-); 3.55 (m, 2H, –NH–CH_2–CH_2-); 4.03 (q, 2H, -O–CH_2-); 7.02–7.25 (m, 4H, Ar); 7.44 (d, 1H, Ar); 7.55 (t, 1H, Ar); 7.71 (d, 1H, Ar); 7.79 (d, 1H, Ar); 8.62 (d, 1H, Ar); 8.96 (t, 1H, –N*H*–CH_2–CH_2-); 11.89 (s, 1H, –NH–); 12.60 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ 14.86, 34.29, 36.38, 60.77, 103.29, 113.20, 120.13, 120.65, 120.89, 122.58, 123.25, 124.76, 127.71, 128.95, 132.36, 133.15, 137.79, 140.11, 159.85, 169.37, 171.88.

6.4.16. 4-{2-[(1*H*-Indole-2-carbonyl)-amino]-benzoylamino}-butyric acid ethyl ester (20c)

Trituration with hot MeOH followed by flash chromatography (CH_2Cl_2 to EtOAc/CH_2Cl_2 1:4) afforded the titled compound in 80% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.59; mp 210 °C; ^{1}H NMR (DMSO-d_6) δ 1.16 (t, 3H, –CH_3); 1.85 (m, 2H, –NH–CH_2–CH_2–CH_2-); 2.41 (t, 2H, –NH–CH_2–CH_2–CH_2–); 3.39 (m, 2H, –NH–CH_2–CH_2–CH_2-); 4.04 (q, 2H, –O–CH_2-); 7.07–7.29 (m, 4H, Ar); 7.49 (d, 1H, Ar); 7.60 (t, 1H, Ar); 7.74 (d, 1H, Ar); 7.88 (d, 1H, Ar); 8.67 (d, 1H, Ar); 8.97 (t, 1H, –N*H*–CH_2–); 11.95 (s, 1H, –NH–), 12.75 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ 14.86, 24.97, 31.81, 60.53, 103.25, 113.20, 120.32, 120.64, 120.87, 122.52, 123.19, 124.71, 127.73, 128.94,132.46, 132.99, 137.82, 140.15, 159.86, 169.35, 173.26.

6.4.17. 3-(*R*,*S*)-{2-[(1H-Indole-2-carbonyl)-amino]-benzoyl-amino}-4-phenyl-butyric acid ethyl ester (21c)

Purification by flash chromatography (CH_2Cl_2 to EtOAc/CH_2Cl_2 1:4) afforded the titled compound in 74% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.57; mp 211 °C; ^{1}H NMR (DMSO-d_6) δ 1.09 (t, 3H –CH_3); 2.64 (m, 2H, –CH_2–CO–); 2.92 (m, 2H, –CH_2–C_6H_5); 3.99 (q, 2H, –CH_2–O–); 4.68 (m, 1H, –CH<); 6.95 (s, 1H, Ar); 7.07–7.27 (m, 8H, Ar); 7.48 (d, 1H, Ar); 7.56 (t, 1H, Ar); 7.72 (m, 2H, Ar); 8.60 (d, 1H, Ar); 8.81 (d, 1H, –N*H*–CH<); 11.89 (s, 1H, –NH–); 12.18 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ 14.18, 48.33, 60.13, 102.79, 112.60, 119.94, 120.22, 120.29, 121.91, 122.57, 124.13, 126.35, 127.07, 128.22, 129.28, 131.66, 132.32, 137.20, 138.41, 139.11, 159.16, 168.02, 170.72.

6.4.18. 2(*S*)-{2-[(1*H*-Indole-2-carbonyl)-amino]-benzoylamino}-4-methyl-pentanoic acid ethyl ester (22c)

Trituration with hot MeOH afforded the titled compound in 62% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.73; $[\alpha]_D^{25}$ = +26.5 (*c* 0.54, CH_2Cl_2); mp 205–206 °C; ^{1}H NMR ($CDCl_3$) δ 0.96 (dd, 6H, –CH(CH_3)$_2$); 1.32 (t, 3H, –CH_2–CH_3); 1.74 (m, 3H, –CH_2–CH(CH_3)$_2$); 4.20 (q, 2H, –CH_2–O–); 4.87 (m, 1H, –NH–CH<); 7.01–7.35 (m, 5H, Ar and –N*H*–CH<); 7.50 (m, 2H, Ar); 7.67 (m, 2H, Ar); 8.80 (d, 1H, Ar); 10.03 (s, 1H, –NH–); 12.20 (s, 1H, –NH–); ^{13}C NMR ($CDCl_3$) δ 14.24, 22.15, 23.01, 25.14, 41.65, 51.37, 61.80, 103.95, 112.15, 119.40, 120.50, 121.25, 122.30, 122.90, 124.75, 127.05, 127.97, 131.48, 133.10, 136.85, 139.90, 160.24, 169.00, 173.20.

6.4.19. 2(*R*)-{2-[(1*H*-Indole-2-carbonyl)-amino]-benzoylamino}-4-methyl-pentanoic acid ethyl ester (23c)

Trituration with hot MeOH afforded the titled compound in 71% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.73; $[\alpha]_D^{25}$ = -24.8 (*c* 0.15, CH_2Cl_2); mp 201–202 °C; ^{1}H NMR ($CDCl_3$) δ 0.95 (dd, 6H, –CH(CH_3)$_2$); 1.30 (t, 3H, –CH_2–CH_3); 1.75 (m, 3H, –CH_2–CH(CH_3)$_2$); 4.25 (q, 2H, –CH_2–O–); 4.90 (m, 1H, –NH–CH<); 7.03–7.33 (m, 5H, Ar and –N*H*–CH<); 7.52 (m, 2H, Ar); 7.66 (m, 2H, Ar); 8.82 (d, 1H, Ar); 10.05 (s, 1H, –NH–); 12.25 (s, 1H, –NH–); ^{13}C NMR ($CDCl_3$) δ 14.22, 22.12, 22.99, 25.18, 41.62, 51.38, 61.82, 103.98, 112.09, 119.44, 120.68, 121.28, 122.30, 122.86, 124.71, 126.92, 127.81, 131.40, 133.08, 136.90, 139.94, 160.13, 168.92, 173.25.

6.4.20. 2(*S*)-{2-[(1*H*-Indole-2-carbonyl)-amino]-benzoylamino}-4-methylsulfanyl-butyric acid ethyl ester (24c)

Crystallization with MeOH afforded the titled compound in 76% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.49; $[\alpha]_D^{25}$ = +48.7 (*c* 0.65, CH_2Cl_2); mp 220–222 °C; ^{1}H NMR (DMSO-d_6) δ 1,29 (t, 3H, –CH_2–CH_3); 2.10 (s, 3H, CH_3–S–); 2.12 (m, 2H, –CH_2–CH<); 2.68 (m, 2H, –CH_2–S–); 4.18 (q, 2H, –CH_2–O–); 4.70 (m, 1H, —CH<); 7.10 (m, 2H, Ar); 7.30 (m, 2H, Ar); 7.50 (d, 1H, Ar); 7.60–7.75 (m, 2H, Ar); 7.98 (d, 1H, Ar); 8.63 (d, 1H, Ar); 9.25 (d, 1H, –N*H*–CH<); 11.90 (s, 1H, –NH–); 12.21 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ 13.84, 14.32, 29.60, 29.71, 51.73, 60.65, 102.39, 112.36, 119.31, 119.87, 120.08, 121.61, 122.42, 123.97, 126.72, 128.53, 131.41, 132.50, 136.90, 138.92, 158.93, 169.17, 171.30.

6.4.21. 2(*R*)-{2-[(1*H*-Indole-2-carbonyl)-amino]-benzoylamino}-4-methylsulfanyl-butyric acid ethyl ester (25c)

Crystallization with MeOH afforded the titled compound in 80% yield. TLC (EtOAc/*n*-hexane 1:1). R_f 0.49; $[\alpha]_D^{25} = -45.8$ (*c* 0.45, CH_2Cl_2); mp 214–215 °C; ^{1}H NMR (DMSO-d_6) δ 1,30 (t, 3H, –CH_2–CH_3); 2.06 (s, 3H, CH_3–S–); 2.10 (m, 2H, –CH_2–CH<); 2.64 (m, 2H, –CH_2–S–); 4.14 (q, 2H, –CH_2–O–); 4.65 (m, 1H, —CH<); 7.07 (m, 2H, Ar); 7.27 (m, 2H, Ar); 7.47 (d, 1H, Ar); 7.56–7.71 (m, 2H, Ar); 7.96 (d, 1H, Ar); 8.64 (d, 1H, Ar); 9.23 (d, 1H, –N*H*–CH<); 11.93 (s, 1H, –NH–); 12.23 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ 13.87, 14.34, 29.60, 29.75, 51.75, 60.62, 102.42, 112.30, 119.31, 119.81, 120.03, 121.60, 122.46, 123.93, 126.74, 128.53, 131.32, 132.44, 136.96, 138.96, 158.91, 169.18, 171.32.

6.5. General procedure for the synthesis of compounds 1–9, 11, 19–21

A mixture of 4.0 mmol of the corresponding ethyl ester (compounds **1c**–**9c**, **11c** and **19c**–**21c**) in water (25 mL) and tetrahydrofuran (25 mL) was stirred in the presence of KOH (0.22 g, 4.0 mmol) at room temperature (under reflux for compound **6**) until completion (TLC monitoring). The organic solvent was removed under reduced pressure and, after cooling to 0 °C, the aqueous solution was adjusted to pH 2–3 with diluted HCl to obtain the precipitation of the corresponding acid.

6.5.1. {2-[(1*H*-Indole-2-carbonyl)-amino]-benzoylamino}-acetic acid (1)

Trituration with hot MeOH afforded the titled compound in 47% yield. TLC (EtOAc/MeOH 3:2). R_f 0.40; mp 264–265 °C; ^{1}H NMR (DMSO-d_6) δ 4.05 (d, 2H, –CH_2-); 7.09 (m, 2H, Ar); 7.24 (m, 2H, Ar); 7.49 (d, 1H, Ar); 7.63 (t, 1H, Ar); 7.75 (d, 1H, Ar); 7.93 (d, 1H, Ar); 8.72 (d, 1H, Ar); 9.32 (t, 1H, –N*H*–CH_2-); 11.95 (s, 1H, –NH–); 12.62 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ 41.12, 102.40, 112.32, 118.63, 118.76, 120.02, 121.71, 122.43, 123.91, 126.81, 128.09, 131.45, 132.56, 136.94, 139.44, 159.02, 168.92, 170.70. MS (ES) *m/z* 336.1 $[MH]^+$; MW: 337.34 (calcd for $C_{18}H_{15}N_3O_4$).

6.5.2. 2-(*R*,*S*)-{2-[(1*H*-Indole-2-carbonyl)-amino]-benzoylamino}-propionic acid (2)

Trituration with hot MeOH afforded the titled compound in 79% yield. TLC (EtOAc/MeOH 2:1). R_f 034; mp 274–276 °C; ^{1}H NMR (DMSO-d_6) δ 1.47 (d, 3H, –CH_3); 4.56 (m, 1H, –CH<); 7.10 (m, 2H, Ar); 7.24 (m, 2H, Ar); 7.50 (d, 1H, Ar); 7.62 (t, 1H, Ar); 7.75 (d, 1H, Ar); 7.97 (d, 1H, Ar); 8.69 (d, 1H, Ar); 9.13 (d, 1H, –N*H*–CH<); 11.95 (s, 1H, –NH–); 12.49 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ 16.46, 48.16, 102.42, 112.33, 119.14, 119.78, 120.02, 121.69, 122.33, 123.89, 126.83, 128.50, 131.48, 132.37, 136.96, 139.22, 159.03, 168.48, 173.62. MS (ES) *m/z* 350.2 $[M-H]^-$, 352.1 $[MH]^+$; MW: 351.37 (calcd for $C_{19}H_{17}N_3O_4$).

6.5.3. 2-(*R*,*S*)-{2-[(1*H*-Indole-2-carbonyl)-amino]-benzoylamino}-3-methyl-butyric acid (3)

Trituration with hot abs. EtOH afforded the titled compound in 86% yield. TLC (EtOAc/MeOH 2:1). R_f 0.58; mp 276 °C; ^{1}H NMR (DMSO-d_6) δ 0.99 (m, 6H, –CH(CH_3)$_2$); 2.25 (m, 1H, –CH(CH_3)$_2$); 4.38 (m, 1H, –NH–CH<); 7.05 (s, 1H, Ar); 7.10 (d, 1H, Ar); 7.25 (m, 2H, Ar); 7.49 (d, 1H, Ar); 7.61 (t, 1H, Ar); 7.73 (d, 1H, Ar); 7.97 (d, 1H, Ar); 8.60 (d, 1H, Ar); 8.92 (d, 1H, –N*H*–CH<); 11.90 (s, 1H, –NH–); 12.23 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ 19.65, 20.08, 30.13, 59.25, 103.27, 113.21, 120.61, 120.70, 120.90, 122.57, 123.30, 124.79, 127.67, 129.75, 132.26, 133.18, 137.81, 139.69, 159.83, 169.79, 173.37. MS (ES) *m/z* 380.0 $[MH]^+$; MW: 379.42 (calcd for $C_{21}H_{21}N_3O_4$).

6.5.4. 2-(*R*,*S*)-{2-[(1*H*-Indole-2-carbonyl)-amino]-benzoylamino}-4-methyl-pentanoic acid (4)

Crystallization with MeOH afforded the titled compound in 23% yield. TLC (EtOAc/MeOH 3:1). R_f 0.51; mp 257–259 °C; ^{1}H NMR (DMSO-d_6) δ (dd, 6H, –CH(CH_3)$_2$); 1.64–1.80 (m, 3H, –CH_2–CH(CH_3)$_2$); 4.56 (m, 1H, –NH–CH<); 7.09 (m, 2H, Ar); 7.25 (m, 2H, Ar); 7.49 (d, 1H, Ar); 7.62 (t, 1H, Ar); 7.74 (d, 1H, Ar); 7.96 (d, 1H, Ar); 8.67 (d, 1H, Ar); 9.07 (d, 1H, –N*H*–CH<); 11.95 (s, 1H, –NH–); 12.43 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ 20.92, 22.78, 24.44, 50.83, 102.35, 112.32, 119.24, 119.79, 120.02, 121.69, 122.38, 124.00, 126.80, 128.61, 131.43, 132.41, 136.94, 139.11,

159.00, 168.80, 173.58. MS (ES) *m/z* 392.1 $[M-H]^-$; MW: 393.45 (calcd for $C_{22}H_{23}N_3O_4$).

6.5.5. 2-(*R,S*)-{2-[(1*H*-Indole-2-carbonyl)-amino]-benzoylamino}-4-methylsulfanyl-butyric acid (5)

Trituration with MeOH afforded the titled compound in 59% yield. TLC (EtOAc/MeOH 2:1). R_f 0.38; mp 250–251 °C; ^{1}H NMR (DMSO-d_6) δ 2.07 (s, 3H, CH_3–S–); 2.14 (m, 2H, –CH_2–CH<); 2.62 (m, 2H, –CH_2–S–); 4.67 (m, 1H, –CH<); 7.09 (m, 2H, Ar); 7.26 (m, 2H, Ar); 7.50 (d, 1H, Ar); 7.63 (t, 1H, Ar); 7.75 (d, 1H, Ar); 7.98 (d, 1H, Ar); 8.69 (d, 1H, Ar); 9.11 (d, 1H, –N*H*—CH<); 11.95 (s, 1H, –NH–); 12.42 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ 14.37, 29.74, 29.97, 51.55, 102.42, 112.33, 119.23, 119.79, 120.03, 121.68, 122.37, 123.91, 126.81, 128.53, 131.44, 132.43, 136.96, 139.13, 159.01, 168.99, 172.89. MS (ES) *m/z* 410.2 $[M-H]^-$, 412.1 $[MH]^+$; MW: 411.48 (calcd for $C_{21}H_{21}N_3O_4S$).

6.5.6. 2-(*R,S*)-{2-[(1-Methyl-1*H*-indole-2-carbonyl)-amino]-benzoylamino}-4-methylsulfanyl-butyric acid (6)

Crystallization with MeOH afforded the titled compound in 60% yield. TLC (EtOAc/MeOH 2:1). R_f 0.48; mp 205–206 °C; ^{1}H NMR (DMSO-d_6) δ 2.05 (s, 3H, CH_3–S–); 2.12 (m, 2H, –CH_2–CH<); 2.60 (m, 2H, –CH_2–S–); 4.06 (s, 3H, >N–CH_3); 4.61 (m, 1H, —CH<); 7.12 (s, 1H, Ar); 7.16–7.30 (m, 2H, Ar); 7.34 (t, 1H, Ar); 7.61 (m, 2H, Ar); 7.75 (d, 1H, Ar); 7.93 (d, 1H, Ar); 8.61 (d, 1H, Ar); 9.08 (d, 1H, –N*H*–CH<); 12.23 (s, 1H, –NH–); 12.90 (b, 1H, -OH); ^{13}C NMR (DMSO-d_6) δ 14.35, 29.72, 29.91, 31.35, 51.49, 104.46, 110.54, 119.75, 119.89, 120.36, 121.75, 122.55, 124.12, 125.26, 128.49, 131.84, 132.29, 138.91, 159.65, 168.90, 172.85. MS (ES) *m/z* 424.2 $[M-H]^-$, 426.1 $[MH]^+$; MW: 425.51 (calcd for $C_{22}H_{23}N_3O_4S$).

6.5.7. 2-(*R,S*)-{2-[(1*H*-Indole-2-carbonyl)-amino]-benzoylamino}-succinic acid (7)

Crystallization with hot abs. EtOH afforded the titled compound in 28% yield. TLC (EtOAc/MeOH 1:1). R_f 0.28; mp 192 °C; ^{1}H NMR (DMSO-d_6) δ 2.82 (m, 2H, –CH_2-); 4.85 (m, 1H, –CH<); 7.08 (m, 2H, Ar); 7.24 (m, 2H, Ar); 7.48 (d, 1H, Ar); 7.62 (t, 1H, Ar); 7.75 (d, 1H, Ar); 7.89 (d, 1H, Ar); 8.69 (d, 1H, Ar); 9.19 (d, 1H, –N*H*–CH<); 11.95 (s, 1H, –NH–); 12.49 (s, 1H, –NH–); 12.87 (b, 2H, –OH); ^{13}C NMR (DMSO-d_6) δ 36.38, 50.13, 103.33, 113.21, 119.70, 120.64, 120.91, 122.64, 123.29, 124.82, 127.68, 129.16, 132.27, 133.46, 137.82, 140.16, 159.86, 169.24, 172.40, 172.85. MS (ES) *m/z* 396.2 $[MH]^+$; MW: 395.37 (calcd for $C_{20}H_{17}N_3O_6$).

6.5.8. 2-(*R,S*)-{2-[(1*H*-Indole-2-carbonyl)-amino]-benzoylamino}-pentanedioic acid (8)

Trituration with hot MeOH afforded the titled compound in 45% yield. TLC (EtOAc/MeOH 1:1). R_f 0.34; mp 239–241 °C; ^{1}H NMR (DMSO-d_6) δ 2.02–2.20 (m, 2H, –CH_2–CH<); 2.42 (m, 2H, –CH_2–CO–); 4.55 (m, 1H, –CH<); 7.07 (m, 2H, Ar); 7.25 (m, 2H, Ar); 7.49 (d, 1H, Ar); 7.63 (t, 1H, Ar); 7.75 (d, 1H, Ar); 7.97 (d, 1H, Ar); 8.70 (d, 1H, Ar); 9.10 (d, 1H, –N*H*–CH<); 11.94 (s, 1H, –NH–); 12.44 (s, 1H, –NH–); 12.57 (b, 2H, –OH); ^{13}C NMR (DMSO-d_6) δ 25.52, 30.23, 51.90, 102.40, 112.32, 119.01, 120.01, 121.72, 122.34, 123.90, 126.81, 128.51, 131.43, 132.48, 136.95, 139.22, 159.01, 168.91, 172.78, 173.66. MS (ES) *m/z* 408.2 $[M-H]^-$; MW: 409.40 (calcd for $C_{21}H_{19}N_3O_6$).

6.5.9. 2-(*R,S*)-{2-[(1*H*-Indole-2-carbonyl)-amino]-benzoylamino}-3-(1*H*-indol-3-yl)-propionic acid (9)

Crystallization with MeOH/1,4-dioxane afforded the titled compound in 68% yield. TLC (EtOAc/MeOH 3:1). R_f 0.44; mp 227–229 °C; ^{1}H NMR (DMSO-d_6) δ 3.38 (m, 2H, –CH_2–CH<); 4.80 (m, 1H, –CH<); 7.01–7.35 (m, 8H, Ar); 7.48–7.76 (m, 4H, Ar); 7.88 (d, 1H, Ar); 8.68 (d, 1H, Ar); 9.13 (d, 1H, –N*H*–CH<); 10.88 (s, 1H, –NH–); 11.94 (s, 1H, –NH–); 12.43 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ 26.32, 53.78, 102.42, 110.19, 11.29, 112.32, 117.96, 118.22, 118.96, 119.65, 120.00, 120.80, 121.74, 122.28, 123.42, 123.89, 126.81, 126.95, 128.35, 131.42, 132.42, 135.94, 136.94, 139.23, 158.98, 168.60, 172.98. MS (ES) *m/z* 465.2 $[M-H]^-$; MW: 466.50 (calcd for $C_{27}H_{22}N_4O_4$).

6.5.10. (2*R,S*)-3-(4-Benzyloxy-phenyl)-2-{2-[(1*H*-indole-2-carbonyl)-amino]-benzoylamino}-propionic acid (11)

Trituration with hot abs. EtOH afforded the titled compound in 76% yield. TLC (EtOAc/MeOH 2:1). R_f 0.59; mp 263–264 °C; ^{1}H NMR (DMSO-d_6) δ 3.18 (m, 2H, –CH_2–CH<); 4.73 (m, 1H, –CH<); 4.91 (m, 2H, -O–CH_2-); 6.86–6.96 (m, 3H, Ar); 7.06–7.17 (m, 2H, Ar); 7.21–7.31 (m, 8H, Ar); 7.47 (d, 1H, Ar); 7.59 (t, 1H, Ar); 7.74 (d, 1H, Ar); 7.83 (d, 1H, Ar); 8.65 (d, 1H, Ar); 9.14 (d, 1H, –NH–CH<); 11.93 (s, 1H, –NH–); 12.26 (s, 1H, –NH–); 12.99 (b, 1H, -OH); ^{13}C NMR (DMSO-d_6) δ 54.32, 68.94, 102.49, 112.42, 114.33, 119.03, 119.67, 120.12, 121.80, 122.38, 123.99, 126.85, 127.56, 128.21, 129.99, 131.42, 132.54, 137.01, 139.18, 152.57, 156.83, 158.96, 168.59, 172.66. MS (ES) *m/z* 532.1 $[M-H]^-$; MW: 533.59 (calcd for $C_{32}H_{27}N_3O_5$).

6.5.11. 3-{2-[(1*H*-Indole-2-carbonyl)-amino]-benzoylamino}-propionic acid (19)

Trituration with hot ethyl acetate afforded the titled compound in 65% yield. TLC (EtOAc/MeOH 2:1). R_f 0.51; mp 277–278 °C; ^{1}H NMR (DMSO-d_6) δ 2.59 (t, 2H, –NH–CH_2–CH_2–); 3.55 (m, 2H, –NH–CH_2–CH_2–); 7.06–7.28 (m, 4H, Ar); 7.48 (d, 1H, Ar); 7.59 (t, 1H, Ar); 7.76 (d, 1H, Ar); 7.85 (d, 1H, Ar); 8.67 (d, 1H, Ar); 8.99 (t, 1H, –N*H*–CH_2); 11.92 (s, 1H, –NH–); 12.33 (b, 1H, –OH); 12.69 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ 34.20, 36.45, 103.31, 113.20, 120.08, 120.61, 120.88, 122.63, 123.22, 124.76, 127.72, 128.99, 132.38, 133.13, 137.79, 140.16, 159.85, 169.34, 173.47. MS (ES) *m/z* 350.1 $[M-H]^-$, 352.0 $[MH]^+$; MW: 351.37 (calcd for $C_{19}H_{17}N_3O_4$).

6.5.12. 4-{2-[(1*H*-Indole-2-carbonyl)-amino]-benzoylamino}-butyric acid (20)

Trituration with hot ethyl acetate afforded the titled compound in 91% yield. TLC (EtOAc/MeOH 2:1). R_f 0.71; mp 258 °C; ^{1}H NMR (DMSO-d_6) δ 1.80 (m, 2H, –NH–CH_2–CH_2–CH_2–); 2.32 (t, 2H, –NH–CH_2–CH_2–CH_2–); 3.36 (m, 2H, –NH–CH_2–CH_2–CH_2–); 7.03–7.26 (m, 4H, Ar); 7.45 (d, 1H, Ar); 7.56 (t, 1H, Ar); 7.72 (d, 1H, Ar); 7.84 (d, 1H, Ar); 8.65 (d, 1H, Ar); 8.92 (t, 1H, –N*H*–CH_2–); 11.89 (s, 1H, –NH–); 12.10 (b, 1H, –OH); 12.68 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ 25.00, 31.88, 103.27, 113.20, 120.28, 120.57, 120.87, 122.61, 123.22, 124.74, 127.72, 128.99, 132.40, 133.03, 137.79, 140.12, 159.84, 169.34, 174.93. MS (ES) *m/z* 364.1 $[M-H]^-$, 366.1 $[MH]^+$; MW: 365.39 (calcd for $C_{20}H_{19}N_3O_4$).

6.5.13. 3-(*R,S*)-{2-[(1*H*-Indole-2-carbonyl)-amino]-benzoylamino}-4-phenyl-butyric acid (21)

Trituration with hot ethyl acetate and then with hot EtOH afforded the titled compound in 54% yield. TLC (EtOAc/MeOH 2:1). R_f 0.70; mp 274 °C; ^{1}H NMR (DMSO-d_6) δ 2.60 (m, 2H, –CH_2–CO-); 2.90 (m, 2H, –CH_2–C_6H_5); 4.66 (m, 1H, –CH<); 6.96 (s, 1H, Ar); 7.07–7.28 (m, 8H, Ar); 7.48 (d, 1H, Ar); 7.56 (t, 1H, Ar); 7.73 (m, 2H, Ar); 8.61 (d, 1H, Ar); 8.78 (d, 1H, –N*H*–CH<); 11.89 (s, 1H, –NH–); 12.23 (s, 1H, –NH–); 12.31 (b, 1H, –OH); ^{13}C NMR (DMSO-d_6) δ 48.25, 102.90, 112.63, 119.90, 120.21, 120.33, 122.02, 122.58, 124.19, 126.34, 127.13, 128.24, 129.32, 131.72, 132.35, 137.23, 138.59, 139.20, 159.23, 168.01, 172.49. MS (ES) *m/z* 440.2 $[M-H]^-$,442.1 $[MH]^+$; MW: 441.49 (calcd for $C_{26}H_{23}N_3O_4$).

6.6. General procedure for the synthesis of compounds 22–25

A mixture of 0.60 mmol of the corresponding ethyl ester (compounds **22c**–**25c**) 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 h. 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 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 1 N HCl. The organic phase was removed under reduced pressure and the product was filtered. Crystalizzation or trituration with MeOH afforded the analytically pure compounds **22–25**.

6.6.1. 2-(*S*)-{2-[(1*H*-Indole-2-carbonyl)-amino]-benzoylamino}-4-methyl-pentanoic acid (22)

Crystallization with MeOH afforded the titled compound in 32% yield. TLC (EtOAc/MeOH 3:1). R_f 0.51; $[\alpha]_D^{25} = -33.2$ (*c* 0.45, DMF); ee > 99.4; mp 263–265 °C; ^{1}H NMR (DMSO-d_6) δ 0.94 (dd, 6H, –CH(CH_3)$_2$); 1.61–1.78 (m, 3H, –CH_2–CH(CH$_3$)$_2$); 4.51 (m, 1H, –NH–CH<); 7.12 (m, 2H, Ar); 7.28 (m, 2H, Ar); 7.51 (d, 1H, Ar); 7.65 (t, 1H, Ar); 7.78 (d, 1H, Ar); 8.01 (d, 1H, Ar); 8.62 (d, 1H, Ar); 9.10 (d, 1H, –NH–CH<); 11.90 (s, 1H, –NH–); 12.40 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ 20.89, 22.73, 24.47, 50.78, 102.31, 112.38, 119.19, 119.72, 120.08, 121.73, 122.32, 124.08, 126.84, 128.66, 131.48, 132.39, 136.90, 139.18, 159.08, 168.84, 173.61. MS (ES) *m/z* 394.3 [MH]$^+$; MW: 393.45 (calcd for $C_{22}H_{23}N_3O_4$).

6.6.2. 2(*R*)-{2-[(1*H*-Indole-2-carbonyl)-amino]-benzoylamino}-4-methyl-pentanoic acid (23)

Crystallization with MeOH afforded the titled compound in 38% yield. TLC (EtOAc/MeOH 3:1). R_f 0.51; $[\alpha]_D^{25} = +36.4$ (*c* 1.2, DMF); ee > 99.7; mp 259–260 °C; ^{1}H NMR (DMSO-d_6) δ 0.96 (dd, 6H, –CH(CH_3)$_2$); 1.62–1.77 (m, 3H, –CH_2–CH(CH$_3$)$_2$); 4.55 (m, 1H, –NH–CH<); 7.10 (m, 2H, Ar); 7.21 (m, 2H, Ar); 7.45 (d, 1H, Ar); 7.60 (t, 1H, Ar); 7.72 (d, 1H, Ar); 7.91 (d, 1H, Ar); 8.64 (d, 1H, Ar); 9.03 (d, 1H, –NH–CH<); 11.91 (s, 1H, –NH–); 12.40 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ 20.89, 22.71, 24.41, 50.86, 102.37, 112.39, 119.21, 119.72, 120.08, 121.63, 122.35, 124.04, 126.83, 128.67, 131.40, 132.44, 136.92, 139.18, 159.08, 168.85, 173.59. MS (ES) *m/z* 394.2 [MH]$^+$; MW: 393.45 (calcd for $C_{22}H_{23}N_3O_4$).

6.6.3. 2(*S*)-{2-[(1*H*-Indole-2-carbonyl)-amino]-benzoylamino}-4-methylsulfanyl-butyric acid (24)

Trituration with MeOH afforded the titled compound in 48% yield. TLC (EtOAc/MeOH 2:1). R_f 0.38; $[\alpha]_D^{25} = -55.3$ (*c* 1.8, DMF); ee > 98.9; mp 253–255 °C; ^{1}H NMR (DMSO-d_6) δ 2.09 (s, 3H, CH$_3$–S–); 2.17 (m, 2H, –CH_2–CH<); 2.68 (m, 2H, –CH$_2$–S–); 4.65 (m, 1H, —CH<); 7.11 (m, 2H, Ar); 7.30 (m, 2H, Ar); 7.54 (d, 1H, Ar); 7.67 (t, 1H, Ar); 7.78 (d, 1H, Ar); 7.94 (d, 1H, Ar); 8.65 (d, 1H, Ar); 9.14 (d, 1H, –NH–CH<); 11.98 (s, 1H, –NH–); 12.46 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ 14.32, 29.71, 29.93, 51.52, 102.46, 112.30, 119.26, 119.81, 120.09, 121.64, 122.32, 123.96, 126.82, 128.58, 131.40, 132.48, 136.92, 139.17, 159.08, 168.93, 172.85. MS (ES) *m/z* 412.2 [MH]$^+$; MW: 411.48 (calcd for $C_{21}H_{21}N_3O_4S$).

6.6.4. 2(*R*)-{2-[(1*H*-Indole-2-carbonyl)-amino]-benzoylamino}-4-methylsulfanyl-butyric acid (25)

Trituration with MeOH afforded the titled compound in 61% yield. TLC (EtOAc/MeOH 2:1). R_f 0.38; $[\alpha]_D^{25} = +54.4$ (*c* 2.1, DMF); ee > 99.6; mp 248–250 °C; ^{1}H NMR (DMSO-d_6) δ 2.06 (s, 3H, CH$_3$–S–); 2.16 (m, 2H, –CH_2–CH<); 2.64 (m, 2H, –CH$_2$–S–); 4.66 (m, 1H, —CH<); 7.10 (m, 2H, Ar); 7.24 (m, 2H, Ar); 7.52 (d, 1H, Ar); 7.65 (t, 1H, Ar); 7.75 (d, 1H, Ar); 7.98 (d, 1H, Ar); 8.64 (d, 1H, Ar); 9.13 (d, 1H, –NH–CH<); 11.96 (s, 1H, –NH–); 12.44 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ 14.34, 29.73, 29.95, 51.53, 102.44, 112.31, 119.24, 119.80, 120.06, 121.66, 122.35, 123.94, 126.80, 128.55, 131.42, 132.45, 136.90, 139.14, 159.05, 168.96, 172.87. MS (ES) *m/z* 412.1 [MH]$^+$; MW: 411.48 (calcd for $C_{21}H_{21}N_3O_4S$).

6.6.5. (2*R*,*S*)-3-(4-Hydroxy-phenyl)-2-{2-[(1*H*-indole-2-carbonyl)-amino]-benzoylamino}-propionic acid (10)

To a solution of 0.400 g (0.641 mmol) of compound **11c** in 150 ml of THF, 40 mg of 10% Pd/C were added and the reaction mixture was stirred for 24 h (TLC control). The reaction mixture was filtered through Celite and evaporated. The residue was purified by flash chromatography (EtOAc to 15% MeOH) followed by trituration with MeOH, obtaining 0.123 g (43%) of a white powder. TLC (EtOAc/MeOH 2:1). R_f 0.69; mp 251–252 °C; ^{1}H NMR (DMSO-d_6) δ 3.10 (m, 2H, –CH$_2$–); 4.64 (m, 1H, –CH<); 6.65 (d, 2H, Ar); 6.99 (s, 1H, Ar); 7.05–7.28 (m, 5H, Ar); 7.46 (d, 1H, Ar); 7.59 (t, 1H, Ar); 7.73 (d, 1H, Ar); 7.83 (d, 1H, Ar); 8.65 (d, 1H, Ar); 9.12 (d, 1H, –NH–CH<); 9.22 (s, 1H, -OH); 11.94 (s, 1H, –NH–); 12.38 (s, 1H, –NH–); 12.95 (b, 1H, –OH); ^{13}C NMR (DMSO-d_6) δ 35.25, 54.51, 102.44, 112.39, 114.90, 118.90, 119.66, 120.07, 121.79, 122.39, 123.96, 126.85, 127.85, 128.34, 129.85, 131.44, 132.54, 136.99, 139.24, 155.71, 158.97, 168.60, 172.69. MS (ES) *m/z* 442.2 [M−H]$^-$, 444.2 [MH]$^+$; MW 443.46 (calcd for $C_{25}H_{21}N_3O_5$).

6.6.6. (1*R*,*S*)-1*H*-Indole-2-carboxylic acid [2-(1-carbamoyl-2-phenyl-ethylcarbamoyl)-phenyl]-amide (12)

Gaseous ammonia was bubbled for 20 min in a stirred suspension of 1.50 g (3.29 mmol) of **14** in 100 mL of MeOH. After stirring for 7 days at rt the product was filtered and purified by trituration with hot EtOH to give 1.20 g (86%) of a white powder. TLC (EtOAc). R_f 0.74; mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 3.11 (m, 2H, –CH$_2$–); 4.77 (m, 1H, –CH<); 6.93 (s, 1H, Ar); 7.02–7.56 (m, 9H, Ar); 7.71 (m, 2H, Ar); 7.81 (d, 1H, Ar); 8.57 (d, 1H, Ar); 8.96 (d, 1H, –NH–CH<); 11.87 (s, 1H, –NH–); 12.24 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ 37.15, 54.74, 102.65, 112.51, 119.75, 119.85, 120.20, 121.81, 122.46, 124.05, 126.25, 126.96, 128.03, 128.55, 129.10, 131.64, 132.36, 137.10, 138.43, 139.19, 159.14, 168.57, 172.86. MS (ES) *m/z* 427.2 [MH]$^+$; MW: 426.48 (calcd for $C_{25}H_{22}N_4O_3$).

6.6.7. (1*R*,*S*)-1*H*-Indole-2-carboxylic acid [2-(1-dipentylcarbamoyl-2-phenyl-ethylcarbamoyl)-phenyl]-amide (13)

To a solution of 1.00 g (2.34 mmol) of **VL-0395** and 0.33 mL (2.34 mmol) of TEA in 50 mL of THF cooled to −20 °C, 0.30 mL (2.34 mmol) of isobutyl chloroformate were added dropwise under stirring. After 30 min 0.96 mL (4.68 mmol) of dipentylamine were added and the reaction mixture was stirred at rt for 18 h. The solvent was evaporated in vacuo and 150 mL of ethyl acetate were added. The organic phase was washed with 1 N NaOH, H_2O, 1 N HCl, H_2O and 5% KCl. After drying on dry Na_2SO_4 the solvent was removed in vacuo and the residue was triturated with hot MeOH and then purified by flash chromatography (CH_2Cl_2 to EtOAc/CH_2Cl_2 1:4). The product was triturated with hot EtOH to give 0.38 g (29%) of a white powder. TLC (EtOAc/*n*-hexane 1:1). R_f 0.26; mp 173 °C; ^{1}H NMR (DMSO-d_6) δ 0.81 (m, 6H, –CH$_3$); 1.14–1.70 (m, 12H, –CH$_2$–); 3.06–3.30 (m, 6H, –CH_2–CH<e –CH$_2$–); 5.11 (m, 1H, –CH<); 6.93 (s, 1H, Ar); 7.06–7.37 (m, 8H, Ar); 7.47 (d, 1H, Ar); 7.59 (m, 2H, Ar); 7.91 (d, 1H, Ar); 8.61 (d, 1H, Ar); 9.27 (d, 1H, –NH–CH<); 11.94 (s, 1H, –NH–); 12.31 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ 14.62, 22.60, 22.68, 27.53, 28.92, 29.22, 38.03, 46.26, 47.81, 52.09, 103.13, 113.30, 119.98, 120.47, 120.99, 122.11, 123.29, 124.83, 127.24, 127.55, 128.89, 129.29, 129.93, 132.25, 133.28, 137.74, 138.32, 139.73, 159.67, 168.98, 170.76. MS (ES) *m/z* 567.2 [MH]$^+$; MW: 566.75 (calcd for $C_{35}H_{42}N_4O_3$).

6.6.8. (1*R*,*S*)-1*H*-Indole-2-carboxylic acid [2-(1-hydroxymethyl-2-phenyl-ethylcarbamoyl)-phenyl]-amide (15)

To a stirred solution of the acid **VL-0395** (0.600 g, 1.40 mmol) in THF/DMF 10/0.3 (10.3 mL) at −10 °C, *N*-methylmorpholine (0.15

mL, 1.40 mmol) was added, followed by ethyl chloroformate (0.13 mL, 1.40 mmol). After 10 min, $NaBH_4$ (0.159 g, 4.20 mmol) was added in one portion. MeOH (15 mL) was then added dropwise to the mixture over a period of 20 min at 0 °C. The solution was stirred for additional 20 min, and then neutralized with 1 N HCl. The organic solvents were removed and the product was extracted with EtOAc (3 × 10 mL). The combined organic phases were washed consecutively with 1 M HCl, H_2O, 5% aq $NaHCO_3$, H_2O, dried (Na_2SO_4) and the solvent was evaporated. Crystallization from EtOAc/petroleum ether 40–60° afforded 0.382 g (66%) of a white powder. TLC (EtOAc/*n*-hexane 1:1). R_f 0.23; mp 228 °C; ^{1}H NMR ($CDCl_3$) δ 2.91 (m, 2H, –C*H*$_2$–C_6H_5); 3.55 (m, 2H,–C*H*$_2$-OH); 4.34 (m, 1H, –CH<); 4.96 (t, 1H, –OH); 6.97 (s, 1H, Ar); 7.04–7.32 (m, 8H, Ar); 7.47–7.60 (m, 2H, Ar); 7.74 (d, 1H, Ar); 7.82 (d, 1H, Ar); 8.63 (m, 2H, –N*H*–CH<e Ar); 11.91 (s, 1H, –NH–); 12.38 (s, 1H, –NH); ^{13}C NMR ($CDCl_3$) δ 37.23, 54.07, 63.66, 103.33, 113.21, 120.49, 120.88, 122.53, 123.13, 124.73, 126.65, 127.68, 128.75, 129.01, 129.76, 132.36, 132.83, 137.78, 139.78, 139.84, 159.77, 169.00. MS (ES) *m/z* 412.2 $[M–H]^-$; MW: 413.48 (calcd for $C_{25}H_{23}N_3O_3$).

7. Molecular modeling

AM1 and conformational search calculations were carried out with the parallel version of the GAUSSIAN03 suite[38] running on an Intel Core™ 2 cpu 6600@2.40 GHz, 3.50 GB RAM, and with the MOPAC2000 semiempirical package[39] on a Kubuntu linux Intel Core™ 2 cpu 6600@2.40 GHz, 3.50 GB RAM. The optimizations were carried out at a gradient norm of 10^{-3} kcal/mol Å, with a tight SCF convergence criterion on the RHF calculations, and using the 'mmok' keyword. The conformational searches were carried out with the Tripos random search[40] and using initially the Tripos forcefield; the geometries yielded by the search were then reoptimized at the AM1 level.

8. 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 (Buckinghamshire, 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.[41]

8.1. [^{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 et al.[20] 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 mM $CaCl_2$, 1% BSA, 0.2 mg/mL Soybean trypsin inhibitor, pH 7.4) was added and the tubes were centrifuged 5 min at 12,500*g*. 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.

8.2. [^{125}I]BH–CCK-8. receptor binding assay in guinea pig cerebral cortices

Membranes from guinea pig cerebral cortices, were prepared as previously described.[20] Protein concentration was determined according to Bradford,[42] 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 1 μM CCK-8, accounting of 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).

8.3. Isolated muscular strips of guinea pig gallbladder stimulated by CCK-8

The assay was based on the method described by Bishop et al.[43] Male Hartley guinea pigs weighting 400 - 450 g (Charles River, Calco, Italy) were sacrificed by cervical dislocation and exsanguination, after slight ethereal anesthesia. The abdomen was opened, the gallbladder was removed and transferred to a Petri dish containing gassed Krebs–Henseleit solution of the following composition (mM): NaCl 118.9, KCl 4.69, $CaCl_2{\cdot}2H_2O$ 3.33, KH_2PO_4 1.18, $MgSO_4{\cdot}7H_2O$ 1.2, $NaHCO_3$ 25, glucose 11.1. The neck and the fundus of the gallbladder was cut away; then the tissue was cut transversally obtaining two rings, each about 3 mm high. From these rings two strips of about 3 × 15 mm were obtained, containing mainly the circular smooth muscle fibers. The strips were suspended with silk thread in 20 ml organ baths containing Krebs–Henseleit solution, maintained at 32°C, bubbled with 95% O_2–5% CO_2. Contractions were measured with an isometric transducer (mod. 7003—Basile U., Comerio, Italy). A resting tension of 0.5 g was applied. The isometric contraction was monitored using a pen recorder (mod. 7070—Basile U., Comerio, Italy). The strips were washed with fresh buffer every 15 min over a 60 min equilibration period.

Submaximal-effect (70–80%) concentration of CCK-8 was chosen to test the inhibitory effects of the substances under study. Various concentrations of the antagonists or vehicles were added, and allowed 5 min contact with the tissues before adding the agonist to the bathing fluid. The antagonist activity was expressed as percentage of inhibition of the agonist contractions. The regression line was calculated and the concentration effective in inhibiting by 50% the effect of the agonist (IC_{50}) were obtained from the regression line by using ALLFIT program.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.02.012.

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