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Anthranilic acid based CCK_1 receptor antagonists: preliminary investigation on their second "touch point"

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Abstract

In this phase of structure–affinity relationship study of **VL-0395**, a new anthranilic acid based CCK_1 selective antagonist, we propose a series of unnatural aminoacidic derivatives. The result of this work is the identification of a new CCK ligand, which possesses an affinity (IC50 = 35 nm) one order of magnitude greater than the lead and, as a general rule, it points out how the hypothesized receptorial pocket which accommodates the Phe residue allows much more structural modification than that interacting with the N-terminal group. Hence, the modification of the C-terminal pharmacophoric group of our lead **VL-0395** can not only enhance the affinity of anthranilic acid derivatives but can modulate the selectivity for one CCK receptor subtype or afford mixed antagonists.

Keywords: Cholecystokinin; CCK_1; Antagonist; Anthranilic acid; Unnatural aminoacids; Receptors

1. Introduction

Cholecystokinin (CCK) is a gastro-intestinal peptide hormone and a neurotransmitter present throughout the nervous system [1]. The hormonal effects of CCK in the gastrointestinal (GI) tract are related to the pancreatic enzyme secretion, gut motility, gall bladder contraction and gastric emptying [2]. In the central nervous system (CNS) there are different CCK-mediated pathways which are involved in a wide variety of physiological and neuropathological processes such as satiety [3], nociception [4], thermoregulation [5], anxiogenesis [6] and panic [7].

The entire range of the biological effects are mediated by two specific receptor subtypes: CCK_1 and CCK_2. The CCK_1 receptor is found abundantly on pancreatic acinar cells; the CCK_2 one, which also functions as the gastrin receptor, is the predominant form in the brain and in the stomach [8].

Until now, much research has been devoted to the search for peptidomimetics as selective ligands of CCK receptors, due to their potential as attractive therapeutic agents. These research efforts have led to the discovery of several classes of non-peptide antagonists which differentially bind the two CCK receptor types [9,10]. 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 ADME/Tox properties (absorption, distribution, metabolism, excretion and toxicity) and for more or less complexity in their synthesis. In the choice of a promising candidate as a lead or for clinical development, all these parameters must be taken into account, in agreement with both definitions of leadlikeness and druglikeness, respectively [11,12].

Our approach to the design of such ligands arose from the disconnection of asperlicin and gave rise to a new class of anthranilic acid based CCK_1 selective antagonists [13]. The obtained lead compound **VL-0395**, characterized by the presence of the 2-indole moiety and the Phe residue, is endowed with good affinity and selectivity towards the CCK_1 receptor and possesses an antagonist nature comparable with that

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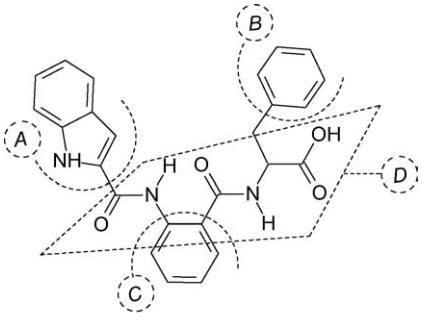
exhibited by the reference CCK_1 selective antagonist Loxiglumide [14].

Considering the two main pharmacophoric groups, we believe that a regnylogical type receptor binding model, requiring at least two hydrophobic pockets, might explain the ligand–receptor interaction. On the contrary, the anthranilic acid does not seem to interact directly with a specific site but is essential for the correct orientation of the two pharmacophoric groups.

Although **VL-0395** is not really a "leadlike" compound according to the definition of Teague et al. [12], in particular for its molecular weight, we consider it an optimal candidate for the application of the well established "step-by-step" lead investigation strategy. In fact, we can envisage the possibility of four independent optimization steps; these include the N- and C-terminal substituent optimization (*A* and *B* in Fig. 1, respectively), the anthranilic acid template and the peptide-like backbone (*C* and *D* in Fig. 1, respectively). Finally, the chiral resolution of the compounds will be considered, in order to verify the stereospecificity of the receptor interaction. Thus, if the assumption that these changes are additive is true, combining the optimal groups at each respective site could provide antagonists with higher affinity and selectivity.

Till now, a structure–affinity relationship study enabled us to demonstrate that the N-terminal substituent highly modulates the affinity and that the 2-indole moiety behaves as a "needle", since any substitution of the indole group with at least 50 different other residues produces a loss in affinity (*A*, Fig. 1) [14,15]. The only modification allowed in this first optimization step is the substitution on the indole ring with groups characterized by minimal steric hindrance, a parameter that deeply influences the affinity. In addition, the replacement of the phenyl ring of the indole moiety with a saturated one—cyclohexyl or cyclopentyl—is detrimental for the affinity, indicating the essential role of the planar aromatic system.

Keeping in mind the difficulties so far encountered, in the current step, directed to the C-terminal optimization of the anthranilic acid template (*B*, Fig. 1), we decided to proceed by a more cautious approach. For this reason, we report on



VL-0395 IC_{50} = 0.197 μM

Fig. 1. Schematic independent "step-by-step" lead optimization strategy of the anthranilic acid derivative **VL-0395**. *A*: N-terminal optimization; *B*: optimization of the aminoacid side chain; *C*: substitution of the anthranilic acid template; *D*: modification of the peptoid backbone.

the series of phenylalanine derivatives depicted in Table 1, where the N-terminus of anthranilic acid was kept constant while different side chains of unnatural aminoacids were introduced at the C-terminal site Table 1.

The chosen substituents on the Phe phenyl ring are characterized by different electronic, hydrophobic and steric properties in order to correlate the biological results to at least one of these variables. In one case the phenyl ring was replaced by a saturated cyclohexyl one, which maintains a similar steric hindrance, to verify if, like for the other receptorial sub-site, the optimal interaction is guaranteed only by an aromatic system.

This first approach will, in the end, clarify if the second considered pharmacophore is as restrictive as the 2-indole moiety, or if more modification is allowed in order to improve this new class of CCK ligands.

2. Chemistry

Compounds **1-20** were synthesized as shown in Fig. 2.

The preparation of the racemic phenylalanine analogues **1c–8c**, **10c–19c** (only 4-fluoro-DL-phenylalanine was purchased from Sigma-Aldrich) proceeds according to the previously described procedure of Snyder et al. [16]. The introduction of the side chain of the aminoacids is achieved reacting the anion of diethyl acetamidomalonate—used as synthon for the aminoacidic portion—with the appropriate benzyl halide. This is a convenient method considering that the benzyl halides used are commercially available, with the exception of bromides **3a**, **5a**, **13a,** which are obtained from the corresponding alcohols by treatment with 48% HBr.

Diethyl α-alkylacetamidomalonate **1b–8b**, **10b–19b** are then hydrolyzed and decarboxylated to aminoacids **1c–8c**, **10c–19c** by refluxing in a 1:1 mixture of 6 N HCl and 1,4-dioxane. Esterification with abs. EtOH saturated with gaseous HCl followed by ortho-amino benzoylation of the DL-phenylalanine ethyl ester derivatives **1d–8d**, **10d–19d** with isatoic anhydride gave intermediates **1e–19e** in good yields.

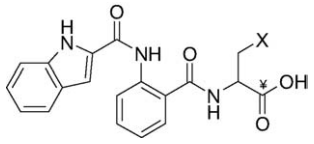
Compounds **1f–19f** were obtained by condensation of anthranoyl derivatives **1e–19e** with indol-2-carboxylic acid activated using PCl_5 in acetyl chloride [17]. The following base catalyzed hydrolysis of the ethyl esters afforded the free acids **1–19** in nearly quantitative yields.

Unfortunately all our attempts to prepare the *o*-amino derivative by reduction of the nitro derivative **16** failed; instead compound **20** was obtained in almost quantitative yield.

3. Results and discussion

Compounds **1–20** were evaluated for their ability to displace [^{125}I] (BH)-CCK8 (sulfated) from isolated rat pancreatic acini (CCK_1) and guinea pig cerebral cortex membranes (CCK_2) according to established protocols [18]. Binding affinities expressed as IC_{50} or as percentage of inhibition (ISB

Table 1
Structure and CCK receptors binding data of the target compounds



Compounds	X	IC_{50}[a] (μM)	
		Rat pancreatic acini (CCK_1)	Guinea pig brain cortex (CCK_2)
VL-0395	Phenyl	0.197 ± 0.107	16.40
1	2-Methyl-phenyl	0.287 ± 0.024	25% ISB[c]
2	4-Methyl-phenyl	0.361 ± 0.119	4.20
3	4-Ethyl-phenyl	1.222 ± 0.302	NT
4	4-Tert-butil-phenyl	1.729 ± 0.415	0.670
5	4-Biphenyl	54% ISB[b]	NT
6	2-Methoxy-phenyl	0.563 ± 0.079	12% ISB[c]
7	3-Methoxy-phenyl	0.298 ± 0.076	18% ISB[c]
8	4-Methoxy-phenyl	0.912 ± 0.141	2.99
9	4-Fluoro–phenyl	0.248 ± 0.050	22% ISB[c]
10	2-Chloro-phenyl	0.510 ± 0.204	22% ISB[c]
11	3-Chloro-phenyl	0.371 ± 0.161	24% ISB[c]
12	4-Chloro-phenyl	0.504 ± 0.176	2.30
13	3,5-Dichloro-phenyl	29% ISB[c]	NT
14	2,6-Dichloro-phenyl	2.017 ± 0.520	19% ISB[c]
15	4-Carboxy-phenyl	12% ISB[c]	NT
16	2-Nitro-phenyl	0.534 ± 0.175	IN[c]
17	4-Nitro-phenyl	0.665 ± 0.211	31% ISB[c]
18	3-Pyridil	0.228 ± 0.043	IN[c]
19	Cyclohexyl	0.035 ± 0.006	1.22
20	2-Amido-phenyl (–NH–¥CO–)	38% ISB[c]	NT

[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. Values without standard errors were obtained from not more than two experiments; IN: inactive; NT: not tested.

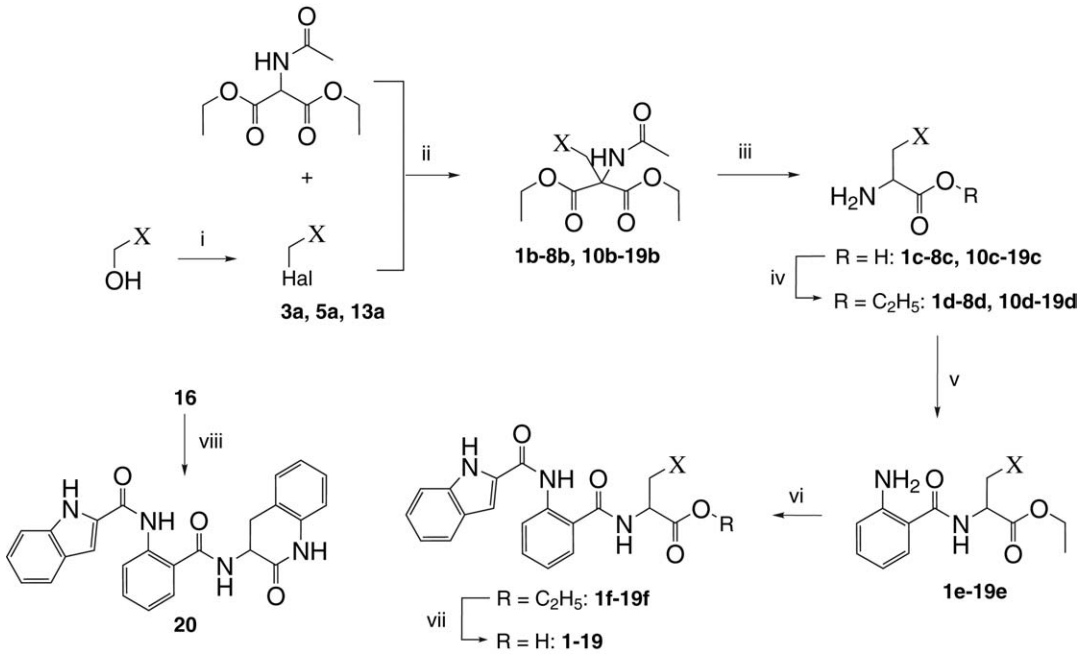


Fig. 2. Preparation of compounds **1**–**20**. Reagents and conditions: (i) HBr 48%, reflux; (ii) EtONa, EtOH abs., reflux; (iii) 6 N HCl, 1,4-dioxane, reflux; (iv) HCl (g), EtOH abs.; (v) isatoic anhydride, Et_3N, AcOEt, reflux; (vi) indol-2-carboxylic acid, PCl_5, dry CH_2Cl_2, pyridine; (vii) KOH, THF/H_2O 1:1; (viii) H_2, 10% Pd/C, THF.

%) determined at the highest used dose (1 μM or 3 μM, as indicated) are reported in Table 1 along with those obtained for the lead compound **VL-0395.** Values without standard errors were obtained from no more than two experiments.

The observed affinities indicate, as expected for this class of compounds, a clear tendency to bind the CCK_1 receptor. When compared with the reference compound **VL-0395,** a wide range of affinities were observed. However, in general, the substitution on the aromatic ring of the C-terminal pharmacophoric group is less significant than that on the N-terminus with analogues of the indole moiety [14,15].

The best affinity is displayed by the cyclohexyl derivative (comp. **19**), the saturated analogue of our lead, which possesses an affinity one order of magnitude greater than that of the reference. On the other hand, within the monosubstituted Phe derivatives, the best results are observed for compounds **1**, **2**, **7**, **9**, **11**, **18** with an affinity similar to that of the lead, even though characterized by quite heterogeneous groups regarding the substituent constants. From these data it could be preliminarily inferred that the affinity is not correlated with either the hydrophobic effect or with the electronic one of the chosen substituents; for example the substituents of compounds **6** and **16** impart similar affinities but have opposite electronic effects.

Moreover, there's not a clear dependency of the affinities on the position of the substituents on the phenyl ring, as shown by the two series of compounds **6–8** and **10–12**.

Actually, it seems that the decreased affinities could be mainly ascribed to the steric effect of the side chain. This consideration is confirmed by the decreased affinities observed for substituents incrementally bulkier (i.e. compounds **4** or **5** in comparison to **2** or **9**) and by the poor affinities exhibited by the disubstituted compounds **13** and **14**.

A conclusive evidence of this macroscopic observation was obtained by correlating the affinities with the molar refractivity (MR), a widely used steric descriptor of the substituent, that takes into account the intermolecular effects -that generally outweigh the intramolecular ones- between a ligand and its receptor.

The data utilized for the regression analysis are reported in Table 2. Regression analysis performed on the homogeneous set of aromatic monosubstituted derivatives produced equation 1.

$$\mathrm{Log}1/\mathrm{IC}_{50} = -0.497(\pm 0.146)\mathrm{MRx} + 7.852(\pm 0.459) \quad (1)$$

$N = 14$, $r = 0.906$, $Q^2 = 0.710$, $s = 0.121$, $F_{1,12} = 54.98$, $\alpha = 0.01$.

MRx, scaled by 0.1, corresponds to the molar refractivity of the phenyl R-substituted substructure ($X = C_6H_4$-R) and was determined using the program C-QSAR by Biobyte [19]. The outlier **7**, marked in Table 2, was not included in this analysis. Being MR a function of volume and polarizability and so mainly a measure of bulkiness, CCK_1 receptor affinity of these derivatives is governed by the overall size of the X-groups, where the negative effect of steric hindrance could be exerted either directly or through a conformational change in the receptor. No significant role for an electronic effect or lipophilicity was found.

Since the calculated MRx has identical values for positional isomers, we tried to elucidate the precise role of the geometrical shape of this pharmacophoric group when holding the same substituent in different positions (Table 3). Thus, Eq. (2) was derived, where Mg_{Vol} is the molar volume of the molecules calculated by the methods of McGowan, $B_5(4)$ and L(2) are Verloop's sterimol parameters [20] for substituent on the aromatic ring. $B_5(4)$ is the measure of the largest width of the first atom of a substituent in position 4, an attempt to define the overall volume and L(2) is the length of substituent in position 2.

$$\mathrm{Log}\ 1/\mathrm{IC}_{50} = -0.006(\pm 0.003)\mathrm{Mg}_{\mathrm{vol}} - 0.135(\pm 0.072)\mathrm{L}(2) - 0.250(\pm 0.059)\mathrm{B}_5(4) + 9.923(\pm 1.243)$$
$$N = 15, r = 0.977, Q^2 = 0.916, s = 0.065, F_{1,13} = 18.139, \alpha = 0.01, F_{1,12} = 31.0, \alpha = 0.01, F_{1,11} = 16.85, \alpha = 0.01, \quad (2)$$
$$F_{3,11} = 38.6, \alpha = 0.01.$$

Eq. (2) is significant in terms of *r* and F and no interrelationship exists among Mg_{Vol}, L(2) and $B_5(4)$. All the terms have a negative sign, indicating unfavorable steric effects for the whole molecule (Mg_{Vol}) as well as for substituents in positions 2 and 4. No specific parameter for substituents in positions 3 has been used; nevertheless the IC_{50} values of compound **7** and **11** are well predicted as well as that of the only pyridyl congener (comp. **18**).

Although this QSAR does not seem to afford much information in terms of ligand–receptor interaction, actually some precious remarks can be stated, based upon the previous equations:

- the bulkiness of the whole residue, and not that of the single substituent, plays an important role in determining the affinity;
- the position of the substituent on the phenyl ring plays only a secondary role;
- no role of electronic effect or lipophilicity is observed.

These results are intriguing, since they are quite different from those obtained in the past for the N-terminal substituent of anthranilic acid. They indicate that the hypothesized receptorial pocket which accommodates the aminoacidic residue is sensitive to steric hindrance but seems to allow many more degrees of freedom than those imposed to the N-terminal group. In fact, although the substitution on the phenyl ring does not improve the affinity, it is really surprising that for the majority of substituted Phe derivatives a good affinity has been observed, being six compounds as active as the lead

Table 2
Data utilized for deriving Eq. (1)

Compounds	X	Obsd. log1/IC_{50} (CCK_1)	Calcd. log1/IC_{50}	Δlog1/IC_{50}	MRx[a]
VL-0395	Phenyl	6.70	6.66	0.04	2.40
1	2-Methyl-phenyl	6.54	6.34	0.20	3.05
2	4-Methyl-phenyl	6.44	6.34	0.10	3.05
3	4-Ethyl-phenyl	5.91	6.11	–0.20	3.51
4	4-Tert-butil-phenyl	5.76	5.65	0.11	4.44
6	2-Methoxy-phenyl	6.25	6.26	–0.01	3.21
7 [b]	3-Methoxy-phenyl	6.52	6.26	0.26	3.21
8	4-Methoxy-phenyl	6.04	6.26	–0.22	3.21
9	4-Fluoro–phenyl	6.60	6.56	0.04	2.60
10	2-Chloro-phenyl	6.29	6.32	–0.03	3.08
11	3-Chloro-phenyl	6.43	6.32	0.11	3.08
12	4-Chloro-phenyl	6.29	6.32	–0.03	3.08
16	2-Nitro-phenyl	6.27	6.26	0.01	3.20
17	4-Nitro-phenyl	6.18	6.26	–0.09	3.20
18	3-Pyridil	6.64	6.67	–0.03	2.37

[a] MRx values are calculated using the C-QSAR program [19].
[b] Data omitted from equation derivation.

Table 3
Data used for deriving Eq. (2)

Compounds[a]	X	Obsd. log1/IC_{50}	Calcd. log1/IC_{50}	Δ log1/IC_{50}	M_{vol}	L(2)	B_5(4)
VL-0395	Phenyl	6.70	6.71	–0.01	427.49	2.06	1.0
1	2-Methyl-phenyl	6.54	6.52	0.02	441.52	2.87	1.0
2	4-Methyl-phenyl	6.44	6.37	0.07	441.52	2.06	2.04
3	4-Ethyl-phenyl	5.91	6.00	–0.09	455.55	2.06	3.17
4	4-Tert-butil-phenyl	5.76	5.82	–0.06	483.61	2.06	3.17
6	2-Methoxy-phenyl	6.25	6.27	–0.02	457.52	3.98	1.0
7	3-Methoxy-phenyl	6.52	6.53	–0.01	457.52	2.06	1.0
8	4-Methoxy-phenyl	6.04	6.01	0.03	457.52	2.06	3.07
9	4-Fluoro–phenyl	6.60	6.51	0.09	445.48	2.06	1.35
10	2-Chloro-phenyl	6.29	6.30	–0.01	461.93	3.52	1.0
11	3-Chloro-phenyl	6.43	6.50	–0.07	461.93	2.06	1.0
12	4-Chloro-phenyl	6.29	6.30	–0.01	461.93	2.06	1.80
16	2-Nitro-phenyl	6.27	6.25	0.02	472.49	3.44	1.0
17	4-Nitro-phenyl	6.18	6.07	0.10	472.49	2.06	2.44
18	3-Pyridil	6.64	6.71	–0.07	428.48	2.06	1.0

[a] Numbers correspond to the corresponding in Table 1.

one. On the other hand, any modification of the N-terminal group, including substitution and particularly saturation, produced a dramatic loss in affinity.

Here, on the contrary, compound **19**, excluded in term of QSAR analysis for its heterogeneous structure, possesses an amazing effect on the CCK_1 receptor.

This result is in agreement with not so strict steric requirements of the receptorial pocket but opens a new possibility of discussion. Why in this case a saturated moiety imparts so a high affinity, while for N-terminal SAfR a quite different situation was observed?

Elucidation of this fact is not unproblematic: in this case the better result obtained by deletion of the π-system implies that the aromatic structure is not crucial for the optimal receptorial recognition process.

A possible explanation could involve an edge-to-face contact between the aminoacidic residue and an aryl residue of the receptorial binding pocket [21,22]. This theory is supported by the consideration that, as evidenced by QSAR data, there's a moderate effect on receptor affinity for substantial changes of the aryl substituting groups, that is the electrostatic potential of the aromatic ring is irrelevant. For the same reason a π stacking interaction can be excluded, for which the electronic term contribution has been well established [21]. A recent study of Wilcox and co-workers [23] highlights how no electronic effect was involved in edge-to-face interactions, suggesting in contrast an important role of London dispersion forces. From this point of view, the higher affinity observed for the cyclohexyl derivative **19** may be consistent with a dominance of dispersion forces in edge-to-face aryl interactions and with multiple C–H contacts involved in CH-π type interactions.

Unfortunately, this hypothesis can not be confirmed, for example, by molecular modeling studies, due to the lack of an appropriate CCK_1 receptorial model, but this interpretation opens further possibility of investigation on this class of CCK antagonists. In this working hypothesis, we believe that the introduction of side chains of unnatural aminoacids, either

aliphatic or aromatic, could provide a modulation of the affinity not observed for the N-terminal modification.

In addition to the results and consequent discussions regarding the CCK_1 receptorial pocket, there is a final point emerging from the data in the table, which opens further working hypothesis: compound **4** is the only one that, despite a not exciting micromolar affinity for CCK_1 receptor, possesses a selectivity, although not so marked, for the CCK_2 receptor subtype. This fact could indicate a new possibility to reverse the selectivity for CCK_2 receptors subtype starting from the same template—anthranilic acid.

Moreover, operating with focused substitution of the C- and N-terminal sites of our lead compound **VL-0395** a modulation of the selectivity of these derivatives for one CCK receptor subtype or mixed antagonists could be obtained.

4. Experimental

4.1. Chemistry

All chemicals and solvents used in syntheses were reagent-grade and were used without additional purification. Reaction progress was monitored by ascending thin-layer chromatography (TLC) using precoated silica gel plates (60F - 254 Merck), visualized by UV light (254 nm). Melting points were determined on a Büchi 510 melting point apparatus (Büchi, Flawil, Switzerland) and are uncorrected. Silica gel (Merck Kieselgel 60, 40–63 μm) was used for flash chromatography. 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 for $CDCl_3$ or $(CD_3)_2SO$ solutions, using Me_4Si as internal standard. Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet and b, broad. Spectral data are consistent with assigned structures. Mass spectra were recorded on an API-1 Perkin-Elmer SCIEX spectrometer by electrospray ionization (ES).

*4.1.1. General procedure for the synthesis of alkyl bromides **3a**, **5a**, **13a***

Ten millimols of the benzyl alcohol were suspended in 25 mL of HBr 48% and heated under reflux for 5 h. The chilled reaction mixture was diluted with H_2O (100 mL) and extracted with diethyl ether (3 × 30 mL). The combined organic phases were washed with a saturated solution of $NaHCO_3$, brine and dried over anhydrous Na_2SO_4. The solvent was rotary evaporated and the crude bromide used without further purification in the next step.

*4.1.2. General procedure for the synthesis of diethyl α-alkyl-acetamidomalonic derivatives **1b–8b**, **10b–19b***

A solution of 20.00 g (92.0 mmol) of diethyl acetamidomalonate and 6.94 g (98.0 mmol) of EtONa 96% was stirred under reflux in 90 mL of abs. EtOH for 30 min. 90.0 mmol of the alkyl halide [24] was then added dropwise and the reaction mixture was refluxed overnight. The mixture was then poured into 500 mL of 0.05 M $KHSO_4$ and 250 mL of n-hexane under vigorous stirring at 0 °C. The product precipitated at the interface was filtered and recrystallized from AcOEt/n-hexane.

The isolation of compound **18b** was achieved as described in literature [25]. The reaction mixture was cooled and the solvent was evaporated in vacuo. 100 mL of $CHCl_3$ were added and the mixture filtered. The organic phase was extracted with 40 mL of 4N HCl, the aqueous phase was brought to pH 6 with 30% NaOH and the product was collected by filtration.

*4.1.2.1. 2-Acetylamino-2-(2-methyl-benzyl)-malonic acid diethyl ester (**1b**).* The product was obtained in 42% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.51; m.p. 83–84 °C (lit. 83–84 °C [26]); ^{1}H-NMR ($CDCl_3$) δ 1.28 (t, 6H, –CH_2–$C\underline{H}_3$); 2.01 (s, 3H, –CO–CH_3); 2.23 (s, 3H, –CH_3); 3.69 (s, 2H, –CH_2–); 4.26 (m, 4H, –CH_2–O–); 6.57 (s, 1H, –NH–); 6.93 (d, 1H, Ar); 7.14 (m, 3H, Ar); ^{13}C-NMR ($CDCl_3$) δ 13.93, 19.29, 23.05, 34.68, 62.57, 66.93, 125.60, 127.12, 130.37, 130.59, 133.45, 137.54, 167.84, 169.14.

*4.1.2.2. 2-Acetylamino-2-(4-methyl-benzyl)-malonic acid diethyl ester (**2b**).* The product was obtained in 45% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.65; m.p. 113 °C (lit. 111–112 °C [27]); ^{1}H-NMR ($CDCl_3$) δ 1.28 (t, 6H, –CH_2–$C\underline{H}_3$); 2.01 (s, 3H, –CH_3); 2.29 (s, 3H, –CO–CH_3); 3.59 (s, 2H, –CH_2–); 4.25 (q, 2H, –CH_2–O–); 6.53 (s, 1H, –NH–); 6.87 (d, 2H, Ar); 7.05 (d, 2H, Ar); ^{13}C-NMR ($CDCl_3$) δ 14.12, 21.16, 23.13, 37.47, 62.68, 67.33, 129.11, 129.77, 132.11, 136.83, 167.64, 169.09.

*4.1.2.3. 2-Acetylamino-2-(4-ethyl-benzyl)-malonic acid diethyl ester (**3b**).* The product was obtained in 98% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.58; m.p. 95 °C; ^{1}H-NMR ($CDCl_3$) δ 1.18 (t, 3H, –CH_2–$C\underline{H}_3$); 1.26 (t, 6H, –O–CH_2–$C\underline{H}_3$); 1.99 (s, 3H, –CO–CH_3); 2.57 (q, 2H, –$C\underline{H}_2$–CH_3); 3.58 (s, 2H, –CH_2–); 4.23 (q, 4H, –CH_2–O–); 6.51 (s, 1H, –NH–); 6.88 (d, 2H, Ar); 7.05 (d, 2H, Ar); ^{13}C-NMR ($CDCl_3$) δ 14.13, 15.48, 23.14, 28.53, 37.55, 62.64, 67.38, 127.82, 129.82, 132.35, 143.06, 167.58, 168.98.

*4.1.2.4. 2-Acetylamino-2-(4-tert-butyl-benzyl)-malonic acid diethyl ester (**4b**).* The product was obtained in 90% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.60; m.p. 82–83 °C; ^{1}H-NMR ($CDCl_3$) δ 1.28 (t, 15H, –$C(CH_3)_3$ and-CH_2–$C\underline{H}_3$); 2.03 (s, 3H, –CO–CH_3); 3.59 (s, 2H, –CH_2–); 4.26 (q, 4H, –CH_2–O–); 6.55 (s, 1H, –NH–); 6.91 (d, 2H, Ar); 7.25 (d, 2H, Ar); ^{13}C-NMR ($CDCl_3$) δ 14.11, 23.14, 31.41, 37.39, 62.69, 67.33, 125.30, 129.60, 132.07, 150.02, 167.66, 169.16.

*4.1.2.5. 2-Acetylamino-2-biphenyl-4-ylmethyl-malonic acid diethyl ester (**5b**).* The product was obtained in 87% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.49; m.p. 138–139 °C (lit.: 138–140 °C [28]); ^{1}H-NMR ($CDCl_3$) δ 1.29 (t, 6H, –CH_2–

$C\underline{H}_3$); 2.03 (s, 3H, –CO–CH_3); 3.68 (s, 2H, –CH_2–); 4.27 (q, 4H, –CH_2–O–); 6.58 (s, 1H, –NH–); 7.06 (d, 2H, Ar); 7.30–7.56 (m, 7H, Ar); ^{13}C-NMR ($CDCl_3$) δ 14.17, 23.17, 37.63, 62.79, 67.37, 127.01, 127.05, 127.32, 128.79, 130.33, 134.34, 140.10, 140.67, 167.54, 169.14.

*4.1.2.6. 2-Acetylamino-2-(2-methoxy-benzyl)-malonic acid diethyl ester (**6b**).* The product was obtained in 80% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.36; m.p. 87–88 °C; ^{1}H-NMR ($CDCl_3$) δ 1.25 (t, 6H, –CH_2–$C\underline{H}_3$); 1.89 (s, 3H, –CO–CH_3); 3.64 (s, 2H, –CH_2–); 3.69 (s, 3H, –O–CH_3); 4.22 (m, 4H, –CH_2–O–); 6.45 (s, 1H, –NH–); 6.80 (m, 2H, Ar); 6.94 (d, 1H, Ar); 7.17 (t, 1H, Ar); ^{13}C-NMR ($CDCl_3$) δ 14.04, 22.90, 32.76, 55.29, 62.37, 66.18, 110.34, 120.39, 123.69, 128.63, 132.21, 157.95, 168.05, 169.02.

*4.1.2.7. 2-Acetylamino-2-(3-methoxy-benzyl)-malonic acid diethyl ester (**7b**).* The product was obtained in 78% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.46; m.p. 86 °C; ^{1}H-NMR ($CDCl_3$) δ 1.24 (t, 6H, –CH_2–$C\underline{H}_3$); 1.98 (s, 3H, –CO–CH_3); 3.57 (s, 2H, –CH_2–); 3.70 (s, 3H, –O–CH_3); 4.22 (q, 4H, –CH_2–O–); 6.55 (m, 3H, Ar and-NH–); 6.74 (d, 1H, Ar); 7.12 (t, 1H, Ar); ^{13}C-NMR ($CDCl_3$) δ 14.09, 23.09, 37.87, 55.11, 62.69, 67.22, 112.43, 115.81, 122.20, 129.30, 136.78, 159.49, 167.51, 169.09.

*4.1.2.8. 2-Acetylamino-2-(4-methoxy-benzyl)-malonic acid diethyl ester (**8b**).* The product was obtained in 72% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.40; m.p. 97 °C (lit. 96–98 °C [29]); ^{1}H-NMR ($CDCl_3$) δ 1.26 (t, 6H, –CH_2–$C\underline{H}_3$); 2.00 (s, 3H, –CO–CH_3); 3.55 (s, 2H, –CH_2–); 3.74 (s, 3H, –O–CH_3); 4.23 (q, 4H, –CH_2–O–); 6.54 (s, 1H, –NH–); 6.76 (d, 2H, Ar); 6.89 (d, 2H, Ar); ^{13}C-NMR ($CDCl_3$) δ 14.10, 23.10, 37.03, 55.20, 62.84, 67.36, 113.78, 127.13, 130.86, 158.78, 167.61, 169.08.

*4.1.2.9. 2-Acetylamino-2-(2-chloro-benzyl)-malonic acid diethyl ester (**10b**).* The product was obtained in 48% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.50; m.p. 93 °C (lit. 94–94.5 °C [30]); ^{1}H-NMR ($CDCl_3$) δ 1.25 (t, 6H, –CH_2–$C\underline{H}_3$); 1.96 (s, 3H, –CO–CH_3); 3.78 (s, 2H, –CH_2–); 4.23 (m, 4H, –CH_2–O–); 6.51 (s, 1H, –NH–); 7.04–7.15 (m, 3H, Ar); 7.29 (m, 1H, Ar); ^{13}C-NMR ($CDCl_3$) δ 13.99, 23.09, 35.15, 62.75, 66.26, 126.61, 128.72, 129.78, 132.31, 133.32, 135.01, 167.65, 169.34.

*4.1.2.10. 2-Acetylamino-2-(3-chloro-benzyl)-malonic acid diethyl ester (**11b**).* The product was obtained in 47% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.58; m.p. 109–110 °C; ^{1}H-NMR ($CDCl_3$) δ 1.27 (t, 6H, –CH_2–$C\underline{H}_3$); 2.02 (s, 3H, –CO–CH_3); 3.60 (s, 2H, –CH_2–); 4.24 (q, 4H, –CH_2–O–); 6.58 (s, 1H, –NH–); 6.88 (m, 1H, Ar); 6.98 (s, 1H, Ar); 7.16 (m, 2H, Ar); ^{13}C-NMR ($CDCl_3$) δ 13.93, 22.89, 37.30, 62.71, 66.93, 127.27, 127.90, 129.44, 129.92, 133.96, 137.22, 167.14, 169.12.

*4.1.2.11. 2-Acetylamino-2-(4-chloro-benzyl)-malonic acid diethyl ester (**12b**).* The product was obtained in 90% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.56; m.p. 142 °C (lit.: 143–144 °C [31]); ^{1}H-NMR ($CDCl_3$) δ 1.25 (t, 6H, –CH_2–$C\underline{H}_3$); 1.99 (s, 3H, –CO–CH_3); 3.58 (s, 2H, –CH_2–); 4.22 (q, 4H, –CH_2–O–); 6.56 (s, 1H, –NH–); 6.90 (d, 2H, Ar); 7.19 (d, 2H, Ar); ^{13}C-NMR ($CDCl_3$) δ 13.75, 22.67, 36.89, 62.48, 66.82, 128.21, 130.94, 132.85, 133.54, 167.05, 169.08.

*4.1.2.12. 2-Acetylamino-2-(3,5-dichloro-benzyl)-malonic acid diethyl ester (**13b**).* The product was obtained in 87% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.60; m.p. 157 °C; ^{1}H-NMR ($CDCl_3$) δ 1.26 (t, 6H, –CH_2–$C\underline{H}_3$); 2.02 (s, 3H, –CO–CH_3); 3.57 (s, 2H, –CH_2–); 4.24 (q, 4H, –CH_2–O–); 6.59 (s, 1H, –NH–); 6.86 (s, 2H, Ar); 7.20 (s, 1H, Ar); ^{13}C-NMR ($CDCl_3$) δ 14.18, 23.12, 37.24, 63.10, 66.97, 127.48, 128.41, 134.76, 138.70, 167.03, 169.41.

*4.1.2.13. 2-Acetylamino-2-(2,6-dichloro-benzyl)-malonic acid diethyl ester (**14b**).* The product was obtained in 42% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.57; m.p. 125–126 °C; ^{1}H-NMR ($CDCl_3$) δ 1.24 (t, 6H, –CH_2–$C\underline{H}_3$); 1.93 (s, 3H, –CO–CH_3); 3.95 (s, 2H, –CH_2–); 4.20 (m, 4H, –CH_2–O–); 6.42 (s, 1H, –NH–); 7.09 (m, 1H, Ar); 7.24 (m, 2H, Ar); ^{13}C-NMR ($CDCl_3$) δ 13.70, 23.10, 33.87, 62.45, 65.17, 128.40, 128.84, 131.74, 136.95, 167.65, 169.16.

*4.1.2.14. 2-Acetylamino-2-(4-ethoxycarbonyl-benzyl)-malonic acid diethyl ester (**15b**).* The product was obtained in 58% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.47; m.p. 138 °C; ^{1}H-NMR ($CDCl_3$) δ 1.24 (t, 6H, –CH_2–$C\underline{H}_3$); 1.32 (t, 3H, –CH_2–$C\underline{H}_3$); 1.98 (s, 3H, –CO–CH_3); 3.65 (s, 2H, –CH_2–); 4.21 (q, 4H, –CH_2–O–); 4.29 (q, 2H, –$C\underline{H}_2$–O–); 6.55 (s, 1H, –NH–); 7.03 (d, 2H, Ar); 7.88 (d, 2H, Ar); ^{13}C-NMR ($CDCl_3$) δ 14.06, 14.36, 23.02, 37.78, 60.96, 62.82, 67.04, 129.53, 129.88, 140.61, 166.31, 167.27, 169.29.

*4.1.2.15. 2-Acetylamino-2-(2-nitro-benzyl)-malonic acid diethyl ester (**16b**).* The product was obtained in 81% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.31; m.p. 102 °C (lit.: 103–104 °C [32]); ^{1}H-NMR ($CDCl_3$) δ 1.24 (t, 6H, –CH_2–$C\underline{H}_3$); 1.93 (s, 3H, –CO–CH_3); 4.03 (s, 2H, –CH_2–); 4.21 (m, 4H, –CH_2–O–); 6.48 (s, 1H, –NH–); 7.21 (d, 1H, Ar); 7.40 (m, 2H, Ar); 7.77 (d, 1H, Ar); ^{13}C-NMR ($CDCl_3$) δ 13.94, 22.86, 34.29, 63.00, 66.35, 124.88, 128.49, 129.83, 132.42, 133.76, 150.51, 167.40, 169.53.

*4.1.2.16. 2-Acetylamino-2-(4-nitro-benzyl)-malonic acid diethyl ester (**17b**).* The product was obtained in 90% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.41; m.p. 188 °C (lit.: 193–194 °C [31]); ^{1}H-NMR ($CDCl_3$) δ 1.27 (t, 6H, –CH_2–$C\underline{H}_3$); 2.02 (s, 3H, –CO–CH_3); 3.73 (s, 2H, –CH_2–); 4.25 (q, 4H, –CH_2–O–); 6.59 (s, 1H, –NH–); 7.15 (d, 2H, Ar); 8.09 (d, 2H, Ar); ^{13}C-NMR ($CDCl_3$) δ 14.10, 23.09, 37.65, 63.12, 66.91, 123.52, 130.79, 143.20, 147.27, 167.10, 169.58.

*4.1.2.17. 2-Acetylamino-2-pyridin-3-yl-methyl-malonic acid diethyl ester (**18b**).* The product was obtained in 20% yield. TLC (AcOEt/MeOH 2:1) - Rf: 0.75; m.p. 103 °C (lit.: 101.5–

102 °C [33]); ^{1}H-NMR ($CDCl_3$) δ 1.30 (t, 6H, –CH_2–$C\underline{H}_3$); 2.06 (s, 3H, –CO–CH_3); 3.67 (s, 2H, –CH_2–); 4.27 (q, 4H, –CH_2–O–); 6.65 (s, 1H, –NH–); 7.24 (m, 1H, Ar); 7.35 (m, 1H, Ar); 8.28 (s, 1H, Ar); 8.48 (m, 1H, Ar); ^{13}C-NMR ($CDCl_3$) δ 14.01, 23.01, 35.12, 62.87, 66.92, 123.14, 130.85, 137.19, 148.53, 150.74, 167.02, 169.22.

*4.1.2.18. 2-Acetylamino-2-cyclohexylmethyl-malonic acid diethyl ester (**19b**).* Trituration with cold hexane afforded the titled compound in 39% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.54; m.p. 77 °C (lit.: 84 °C [34]); ^{1}H-NMR ($CDCl_3$) δ 0.93–1.13 (m, 6H, cyclohexyl); 1.22 (t, 6H, –CH_2–$C\underline{H}_3$); 1.50–1.57 (m, 5H, cyclohexyl); 2.01 (s, 3H, –CO–CH_3); 2.26 (d, 2H, –$C\underline{H}_2$–C_6H_{11}); 4.19 (q, 4H, –CH_2–O–); 6.84 (s, 1H, –NH–); ^{13}C-NMR ($CDCl_3$) δ 14.03, 23.17, 26.13, 26.22, 33.44, 33.85, 38.83, 62.51, 65.95, 168.73, 168.93.

*4.1.3. General procedure for the synthesis of aminoacids hydrochloride **1c–8c**, **10c–19c***

A suspension of 0.025 mol of diethyl α-alkyl-acetamidomalonate in 150 mL of a solution 1:1 of 6 N HCl and dioxane was stirred under reflux. After completion (TLC monitoring), the solvent was removed by evaporation under reduced pressure and the residue was triturated with cold diethyl ether. The product was collected by filtration.

*4.1.3.1. 2-Amino-3-o-tolyl-propionic acid hydrochloride (**1c**).* The product was obtained in 65% yield. TLC (n-BuOH/AcOH/H_2O 3:1:1) - Rf: 0.64; m.p. 218–219 °C; ^{1}H-NMR (DMSO-d_6) δ 2.29 (s, 3H, –CH_3); 3.13 (m, 2H, –CH_2–); 3.92 (m, 1H, –CH<); 7.20 (m, 4H, Ar); 8.79 (b, 3H, –NH_3^+); ^{13}C-NMR (DMSO-d_6) δ 19.23, 33.66, 52.64, 126.03, 127.24, 130.17, 130.37, 133.74, 136.65, 170.42.

*4.1.3.2. 2-Amino-3-p-tolyl-propionic acid hydrochloride (**2c**).* The product was obtained in 83% yield. TLC (n-BuOH/AcOH/H_2O 3:1:1) - Rf: 0.70; m.p. 268 °C; ^{1}H-NMR (DMSO-d_6) δ 2.27 (s, 3H, –CH_3); 3.13 (m, 2H, –CH_2–); 4.07 (m, 1H, –CH<); 7.15 (m, 4H, Ar); 8.60 (b, 3H, –NH_3^+); ^{13}C-NMR (DMSO-d_6) δ 20.51, 34.97, 53.06, 128.86, 129.22, 131.66, 135.96, 169.97.

*4.1.3.3. 2-Amino-3-(4-ethyl-phenyl)-propionic acid hydrochloride (**3c**).* The product was obtained in 82% yield. TLC (n-BuOH/AcOH/H_2O 3:1:1) - Rf: 0.35; m.p. 247 °C; ^{1}H-NMR (DMSO-d_6) δ 1.11 (t, 3H, –CH_2–$C\underline{H}_3$); 2.52 (q, 2H, –$C\underline{H}_2$–CH_3); 3.10 (m, 2H, –$C\underline{H}_2$–CH<); 4.03 (m, 1H, –CH<); 7.13 (m, 4H, Ar); 8.60 (b, 3H, –NH_3^+); ^{13}C-NMR (DMSO-d_6) δ 16.34, 28.58, 35.93, 54.00, 128.55, 130.18, 132.88, 143.10, 170.85.

*4.1.3.4. 2-Amino-3-(4-tert-butyl-phenyl)-propionic acid hydrochloride (**4c**).* The product was obtained in 70% yield. TLC (n-BuOH/AcOH/H_2O 3:1:1) - Rf: 0.73; m.p. 228–232 °C; ^{1}H-NMR (DMSO-d_6) δ 1.29 (m, 9H, –$C(CH_3)_3$); 3.10 (m, 2H, –CH_2–); 4.15 (m, 1H, –CH<); 7.22 (d, 2H, Ar); 7.37 (d, 2H, Ar); 8.44 (b, 3H, –NH_3^+); ^{13}C-NMR (DMSO-d_6) δ 30.97, 35.03, 52.95, 125.11, 129.01, 131.65, 149.22, 170.15.

*4.1.3.5. 2-Amino-3-biphenyl-4-yl-propionic acid hydrochloride (**5c**).* The product was obtained in 85% yield. TLC (n-BuOH/AcOH/H_2O 3:1:1) - Rf: 0.30; m.p. 256–258 °C; ^{1}H-NMR (DMSO-d_6) δ 3.21 (m, 2H, –CH_2–); 4.14 (m, 1H, –CH<); 7.41 (m, 5H, Ar); 7.58 (m, 4H, Ar); 8.66 (b, 3H, –NH_3^+); ^{13}C-NMR (DMSO-d_6) δ 35.95, 53.93, 127.23, 127.43, 128.12, 129.67, 130.91, 135.00, 139.60, 140.43, 170.86.

*4.1.3.6. 2-Amino-3-(2-methoxy-phenyl)-propionic acid hydrochloride (**6c**).* The product was obtained in 86% yield. TLC (n-BuOH/AcOH/H_2O 3:1:1) - Rf: 0.63; m.p. 185–186 °C; ^{1}H-NMR (DMSO-d_6) δ 3.10 (m, 2H, –CH_2–); 3.76 (s, 3H, –O–CH_3); 3.98 (m, 1H, –CH<); 6.87 (t, 1H, Ar); 6.96 (d, 1H, Ar); 7.21 (m, 2H, Ar); 8.50 (b, 3H, –NH_3^+); ^{13}C-NMR (DMSO-d_6) δ 30.95, 51.87, 55.09, 110.57, 120.07, 122.58, 128.61, 130.87, 157.32, 170.04.

*4.1.3.7. 2-Amino-3-(3-methoxy-phenyl)-propionic acid hydrochloride (**7c**).* The product was obtained in 60% yield. TLC (n-BuOH/AcOH/H_2O 3:1:1) - Rf: 0.61; m.p. 190–191 °C; ^{1}H-NMR (DMSO-d_6) δ 3.17 (m, 2H, –CH_2–); 3.73 (s, 3H, –O–CH_3); 4.12 (m, 1H, –CH<); 6.83–6.93 (m, 3H, Ar); 7.21 (t, 1H, Ar); 8.54 (b, 3H, –NH_3^+); ^{13}C-NMR (DMSO-d_6) δ 35.61, 53.27, 55.09, 112.79, 115.32, 121.78, 129.61, 136.56, 159.33, 170.19.

*4.1.3.8. 2-Amino-3-(4-methoxy-phenyl)-propionic acid hydrochloride (**8c**).* The product was obtained in 75% yield. TLC (n-BuOH/AcOH/H_2O 3:1:1) - Rf: 0.59; m.p. 219–220 °C; ^{1}H-NMR (DMSO-d_6) δ 3.12 (m, 2H, –CH_2–); 3.71 (s, 3H, –O–CH_3); 4.05 (m, 1H, –CH<); 6.87 (d, 2H, Ar); 7.21 (d, 2H, Ar); 8.61 (b, 3H, –NH_3^+); ^{13}C-NMR (DMSO-d_6) δ 34.76, 53.43, 55.12, 113.95, 126.80, 130.69, 158.44, 170.21.

*4.1.3.9. 2-Amino-3-(2-chloro-phenyl)-propionic acid hydrochloride (**10c**).* The product was obtained in 89% yield. TLC (n-BuOH/AcOH/H_2O 3:1:1) - Rf: 0.67; m.p. 251–252 °C; ^{1}H-NMR (DMSO-d_6) δ 3.29 (m, 2H, –CH_2–); 4.02 (m, 1H, –CH<); 7.27–7.45 (m, 4H, Ar); 8.81 (b, 3H, –NH_3^+); ^{13}C-NMR (DMSO-d_6) δ 33.80, 51.92, 127.43, 129.19, 129.41, 132.04, 133.14, 133.55, 169.97.

*4.1.3.10. 2-Amino-3-(3-chloro-phenyl)-propionic acid hydrochloride (**11c**).* The product was obtained in 89% yield. TLC (n-BuOH/AcOH/H_2O 3:1:1) - Rf: 0.68; m.p. 245–246 °C; ^{1}H-NMR (DMSO-d_6) δ 3.17 (m, 2H, –CH_2–); 4.14 (m, 1H, –CH<); 7.32 (m, 4H, Ar); 8.59 (b, 3H, –NH_3^+); ^{13}C-NMR (DMSO-d_6) δ 35.15, 53.08, 127.33, 128.50, 129.57, 130.47, 133.16, 137.72, 170.14.

*4.1.3.11. 2-Amino-3-(4-chloro-phenyl)-propionic acid hydrochloride (**12c**).* The product was obtained in 56% yield. TLC (n-BuOH/AcOH/H_2O 3:1:1) - Rf: 0.67; m.p. 254–255 °C;

^{1}H-NMR (DMSO-d_6) δ 3.17 (m, 2H, –CH_2–); 4.13 (m, 1H, –CH<); 7.35 (m, 4H, Ar); 8.54 (b, 3H, –NH_3^+); ^{13}C-NMR (DMSO-d_6) δ 34.84, 53.11, 128.50, 131.55, 131.96, 134.18, 170.09.

*4.1.3.12. 2-Amino-3-(3,5-dichloro-phenyl)-propionic acid hydrochloride (**13c**).* The product was obtained in 69% yield. TLC (n-BuOH/AcOH/H_2O 3:1:1) - Rf: 0.41; m.p. 224–225 °C; ^{1}H-NMR (DMSO-d_6) δ 3.16 (m, 2H, –CH_2–); 4.20 (m, 1H, –CH<); 7.37 (s, 2H, Ar); 7.47 (s, 1H, Ar); 8.60 (b, 3H, –NH_3^+); ^{13}C-NMR (DMSO-d_6) δ 35.30, 53.38, 127.57, 129.24, 134.59, 140.13, 170.61.

*4.1.3.13. 2-Amino-3-(2,6-dichloro-phenyl)-propionic acid hydrochloride (**14c**).* The product was obtained in 84% yield. TLC (n-BuOH/AcOH/H_2O 3:1:1) - Rf: 0.67; m.p. 224–225 °C; ^{1}H-NMR (DMSO-d_6) δ 3.46 (m, 2H, –CH_2–); 3.96 (m, 1H, –CH<); 7.29–7.55 (m, 3H, Ar); 8.91 (b, 3H, –NH_3^+); ^{13}C-NMR (DMSO-d_6) δ 31.77, 50.19, 128.45, 129.88, 131.48, 135.35, 169.54.

*4.1.3.14. 4-(2-Amino-2-carboxy-ethyl)-benzoic acid ethyl ester hydrochloride (**15c**).* The product was obtained in 77% yield. ^{1}H-NMR (DMSO-d_6) δ 3.27 (m, 2H, –CH_2–); 4.21 (m, 1H, –CH<); 7.45 (d, 2H, Ar); 7.91 (d, 2H, Ar); 8.68 (b, 3H, –NH_3^+); ^{13}C-NMR (DMSO-d_6) δ 35.61, 53.09, 129.61, 129.81, 129.93, 140.42, 167.29, 170.15.

*4.1.3.15. 2-Amino-3-(2-nitro-phenyl)-propionic acid hydrochloride (**16c**).* The product was obtained in 89% yield. TLC (n-BuOH/AcOH/H_2O 3:1:1) - Rf: 0.58; m.p. 213–214 °C; ^{1}H-NMR (DMSO-d_6) δ 3.52 (m, 2H, –CH_2–); 4.12 (m, 1H, –CH<); 7.55 (d, 1H, Ar); 7.70 (m, 2H, Ar); 8.05 (d, 1H, Ar); 8.81 (b, 3H, –NH_3^+); ^{13}C-NMR (DMSO-d_6) δ 32.86, 52.69, 125.01, 128.96, 130.62, 133.34, 133.94, 149.13, 169.95.

*4.1.3.16. 2-Amino-3-(4-nitro-phenyl)-propionic acid hydrochloride (**17c**).* The product was obtained in 77% yield. TLC (n-BuOH/AcOH/H_2O 3:1:1) - Rf: 0.64; m.p. 237 °C; ^{1}H-NMR (DMSO-d_6) δ 3.33 (m, 2H, –CH_2–); 4.23 (m, 1H, –CH<); 7.60 (d, 2H, Ar); 8.15 (d, 2H, Ar); 8.74 (b, 3H, –NH_3^+); ^{13}C-NMR (DMSO-d_6) δ 34.97, 52.55, 123.26, 130.79, 143.29, 146.48, 169.64.

*4.1.3.17. 2-Amino-3-pyridin-3-yl-propionic acid hydrochloride (**18c**).* The product, a hygroscopic solid, was obtained in 97% yield. ^{1}H-NMR (DMSO-d_6) δ 3.43 (m, 2H, –CH_2–); 4.36 (m, 1H, –CH<); 8.04 (t, 1H, Ar); 8.57 (d, 1H, Ar); 8.82 (m, 4H, Ar and –NH_3^+); 8.92 (s, 1H, Ar); ^{13}C-NMR (DMSO-d_6) δ 32.79, 53.02, 127.74, 136.45, 140.75, 142.86, 147.90, 170.23.

*4.1.3.18. 2-Amino-3-cyclohexyl-propionic acid hydrochloride (**19c**).* The product was obtained in 65% yield. TLC (n-BuOH/AcOH/H_2O 3:1:1) - Rf: 0.85; m.p. 259 °C dec.; ^{1}H-NMR (DMSO-d_6) δ 0.86 (m, 2H, cyclohexyl); 1.16 (m, 3H, cyclohexyl); 1.51–1.68 (m, 8H, cyclohexyl and –C<u>H</u>$_2$–C_6H_{11}); 3.82 (m, 1H, >C<u>H</u>–CH_2–C_6H_{11});); 8.49 (b, 3H, –NH_3^+); ^{13}C-NMR (DMSO-d_6) δ 25.20, 25.37, 25.65, 31.96, 32.25, 32.44, 37.44, 49.61, 171.21.

*4.1.4. General procedure for the synthesis of aminoacid ethyl esters hydrochloride **1d–8d**, **10d–19d***

Gaseous HCl is bubbled for 30 min. into a stirred suspension of 20.0 mmol of the aminoacid hydrochloride in 150 mL of abs. EtOH, cooled to 0 °C. In the case of compounds **5d**, **10d, 16d** and **18d** the reaction mixture is then heated under reflux for 2 h. After evaporation of the solvent, the residue is triturated with cold diethyl ether and filtered.

*4.1.4.1. 2-Amino-3-o-tolyl-propionic acid ethyl ester hydrochloride (**1d**).* The product was obtained in 97% yield. TLC (AcOEt/MeOH 1:1) - Rf: 0.79; m.p. 88–89 °C; ^{1}H-NMR (DMSO-d_6) δ 0.92 (t, 3H, –CH_2–C<u>H</u>$_3$); 2.30 (s, 3H, –CH_3); 3.20 (m, 2H, –C<u>H</u>$_2$–CH<); 3.95 (m, 3H, –CH< and-CH_2–O–); 7.13 (m, 4H, Ar); 9.02 (b, 3H, –NH_3^+); ^{13}C-NMR (DMSO-d_6) δ 13.60, 19.04, 33.90, 52.43, 61.38, 125.93, 127.27, 130.05, 130.28, 133.36, 136.43, 169.06.

*4.1.4.2. 2-Amino-3-p-tolyl-propionic acid ethyl ester hydrochloride (**2d**).* The product was obtained in 87% yield. TLC (AcOEt/MeOH 1:1) - Rf: 0.67; m.p. 175 °C; ^{1}H-NMR (DMSO-d_6) δ 1.08 (t, 3H, –CH_2–C<u>H</u>$_3$); 2.26 (s, 3H, –CH_3); 3.12 (m, 2H, –C<u>H</u>$_2$–CH<); 4.09 (m, 3H, –CH< and-CH_2–O–); 7.12 (m, 4H, Ar); 8.85 (b, 3H, –NH_3^+); ^{13}C-NMR (DMSO-d_6) δ 13.62, 20.50, 35.28, 53.10, 61.29, 128.85, 129.13, 131.45, 136.05, 168.66.

*4.1.4.3. 2-Amino-3-(4-ethyl-phenyl)-propionic acid ethyl ester hydrochloride (**3d**).* The product was obtained in 95% yield. TLC (AcOEt/MeOH 1:1) - Rf: 0.84; m.p. 155–156 °C; ^{1}H-NMR (DMSO-d_6) δ 1.04 (t, 3H, –CH_3); 1.12 (t, 3H, –CH_3); 2.55 (q, 2H, –C<u>H</u>$_2$–CH_3); 3.08 (m, 2H, –C<u>H</u>$_2$–CH<); 4.06 (m, 3H, –CH< and-CH_2–O–); 7.12 (m, 4H, Ar); 8.73 (b, 3H, –NH_3^+); ^{13}C-NMR (DMSO-d_6) δ 14.54, 16.43, 28.59, 36.32, 53.99, 62.26, 128.59, 130.09, 132.56, 143.33, 169.61.

*4.1.4.4. 2-Amino-3-(4-tert-butyl-phenyl)-propionic acid ethyl ester hydrochloride (**4d**).* The product was obtained in 85% yield. TLC (AcOEt/MeOH 1:1) - Rf: 0.81; m.p. 152 °C; ^{1}H-NMR (DMSO-d_6) δ 1.09 (t, 3H, –CH_2–C<u>H</u>$_3$); 1.27 (m, 9H, –$C(CH_3)_3$); 3.06 (m, 2H, –C<u>H</u>$_2$–CH<); 4.12 (q, 2H, –CH_2–O–); 4.29 (m, 1H, –CH<); 7.16 (d, 2H, Ar); 7.36 (d, 2H, Ar); 8.42 (b, 3H, –NH_3^+); ^{13}C-NMR (DMSO-d_6) δ 13.53, 30.94, 34.02, 35.43, 52.99, 61.48, 125.13, 128.92, 131.24, 149.45, 168.86.

*4.1.4.5. 2-Amino-3-biphenyl-4-yl-propionic acid ethyl ester hydrochloride (**5d**).* The product was obtained in 97% yield. TLC (AcOEt/MeOH 1:1) - Rf: 0.82; m.p. 202–203 °C; ^{1}H-NMR (DMSO-d_6) δ 1.05 (t, 3H, –CH_3); 3.20 (m, 2H, –C<u>H</u>$_2$–CH<); 4.07 (q, 2H, –CH_2–O–); 4.19 (m, 1H, –CH<);

7.31–7.45 (m, 5H, Ar); 7.60 (m, 4H, Ar); 8.88 (b, 3H, $-NH_3^+$); ^{13}C-NMR (DMSO-d_6) δ 14.58, 36.25, 53.93, 62.32, 127.22, 127.42, 128.13, 129.66, 130.81, 134.77, 139.69, 140.41, 169.55.

*4.1.4.6. 2-Amino-3-(2-methoxy-phenyl)-propionic acid ethyl ester hydrochloride (**6d**).* The product was obtained in 86% yield. TLC (AcOEt/MeOH 1:1) - Rf: 0.76; m.p. 113–114 °C; ^{1}H-NMR (DMSO-d_6) δ 0.99 (t, 3H, $-CH_2-C\underline{H}_3$); 3.14 (m, 2H, $-C\underline{H}_2-CH<$); 3.76 (s, 3H, $-O-CH_3$); 3.98 (m, 3H, –CH< and-CH_2–O–); 6.87 (t, 1H, Ar); 6.97 (d, 1H, Ar); 7.17 (d, 1H, Ar); 7.26 (t, 1H, Ar); 8.88 (b, 3H, $-NH_3^+$); ^{13}C-NMR (DMSO-d_6) δ 13.45, 31.11, 51.76, 55.13, 61.07, 110.43, 120.09, 122.44, 128.64, 130.79, 157.23, 168.76.

*4.1.4.7. 2-Amino-3-(3-methoxy-phenyl)-propionic acid ethyl ester hydrochloride (**7d**).* The product was obtained in 94% yield. TLC (AcOEt/MeOH 1:1) - Rf: 0.77; m.p. 113–114 °C; ^{1}H-NMR (DMSO-d_6) δ 1.09 (t, 3H, $-CH_2-C\underline{H}_3$); 3.16 (m, 2H, $-C\underline{H}_2-CH<$); 3.73 (s, 3H, $-O-CH_3$); 4.14 (m, 3H, –CH< and-CH_2–O–); 6.79–6.88 (m, 3H, Ar); 7.22 (t, 1H, Ar); 8.89 (b, 3H, $-NH_3^+$); ^{13}C-NMR (DMSO-d_6) δ 13.60, 35.61, 52.96, 54.80, 61.32, 112.59, 114.83, 121.37, 129.33, 136.04, 159.04, 168.62.

*4.1.4.8. 2-Amino-3-(4-methoxy-phenyl)-propionic acid ethyl ester hydrochloride (**8d**).* The product was obtained in 91% yield. TLC (AcOEt/MeOH 1:1) - Rf: 0.72; m.p. 152–153 °C; ^{1}H-NMR (DMSO-d_6) δ 1.08 (t, 3H, $-CH_2-C\underline{H}_3$); 3.14 (m, 2H, $-C\underline{H}_2-CH<$); 3.71 (s, 3H, –O–CH_3); 4.07 (m, 3H, –CH< and-CH_2–O–); 6.86 (d, 2H, Ar); 7.19 (d, 2H, Ar); 8.77 (b, 3H, $-NH_3^+$); ^{13}C-NMR (DMSO-d_6) δ 13.83, 35.03, 53.43, 55.10, 61.49, 113.91, 126.55, 130.57, 158.45, 168.88.

*4.1.4.9. 2-Amino-3-(2-chloro-phenyl)-propionic acid ethyl ester hydrochloride (**10d**).* The product was obtained in 92% yield. TLC (AcOEt/MeOH 1:1) - Rf: 0.78; m.p. 99–100 °C; ^{1}H-NMR (DMSO-d_6) δ 0.96 (t, 3H, $-CH_3$); 3.37 (m, 2H, $-C\underline{H}_2-CH<$); 4.01 (m, 3H, –CH< and-CH_2–O–); 7.28–7.47 (m, 4H, Ar); 9.03 (b, 3H, $-NH_3^+$); ^{13}C-NMR (DMSO-d_6) δ 13.54, 33.76, 51.89, 61.57, 127.44, 129.24, 129.29, 131.92, 132.89, 133.43, 168.60.

*4.1.4.10. 2-Amino-3-(3-chloro-phenyl)-propionic acid ethyl ester hydrochloride (**11d**).* The product was obtained in 92% yield. TLC (AcOEt/MeOH 1:1) - Rf: 0.78; m.p. 140–141 °C; ^{1}H-NMR (DMSO-d_6) δ 0.98 (t, 3H, $-CH_3$); 3.18 (m, 2H, $-C\underline{H}_2-CH<$); 4.05 (m, 3H, –CH< and-CH_2–O–); 7.26 (m, 4H, Ar); 8.91 (b, 3H, $-NH_3^+$); ^{13}C-NMR (DMSO-d_6) δ 13.78, 35.25, 53.00, 61.59, 127.16, 128.26, 129.42, 130.29, 133.00, 137.53, 168.66.

*4.1.4.11. 2-Amino-3-(4-chloro-phenyl)-propionic acid ethyl ester hydrochloride (**12d**).* The product was obtained in 93% yield. TLC (AcOEt/MeOH 1:1) - Rf: 0.78; m.p. 158 °C; ^{1}H-NMR (DMSO-d_6) δ 1.09 (t, 3H, $-CH_3$); 3.20 (m, 2H, $-C\underline{H}_2-CH<$); 4.10 (m, 3H, –CH< and-CH_2–O–); 7.34 (m, 4H, Ar); 8.88 (b, 3H, $-NH_3^+$); ^{13}C-NMR (DMSO-d_6) δ 13.79, 35.01, 53.03, 61.60, 128.41, 131.42, 131.91, 133.95, 168.68.

*4.1.4.12. 2-Amino-3-(3,5-dichloro-phenyl)-propionic acid ethyl ester hydrochloride (**13d**).* The product was obtained in 92% yield. TLC (AcOEt/MeOH 1:1) - Rf: 0.88; m.p. 153–154 °C; ^{1}H-NMR (DMSO-d_6) δ 1.08 (t, 3H, $-CH_3$); 3.18 (m, 2H, $-C\underline{H}_2-CH<$); 4.08 (m, 2H, $-CH_2-O-$); 4.25 (m, 1H, –CH<); 7.36 (s, 2H, Ar); 7.45 (s, 1H, Ar); 8.87 (b, 3H, $-NH_3^+$); ^{13}C-NMR (DMSO-d_6) δ 14.59, 35.46, 53.40, 62.48, 127.53, 129.22, 134.57, 140.03, 169.18.

*4.1.4.13. 2-Amino-3-(2,6-dichloro-phenyl)-propionic acid ethyl ester hydrochloride (**14d**).* The product was obtained in 93% yield. TLC (AcOEt/MeOH 1:1) - Rf: 0.87; m.p. 159–160 °C; ^{1}H-NMR (DMSO-d_6) δ 0.91 (t, 3H, $-CH_3$); 3.46 (m, 2H, $-C\underline{H}_2-CH<$); 3.98 (m, 3H, –CH< and-CH_2–O–); 7.33–7.52 (m, 3H, Ar); 9.05 (b, 3H, $-NH_3^+$); ^{13}C-NMR (DMSO-d_6) δ 13.30, 31.66, 49.93, 61.88, 128.50, 130.05, 131.23, 135.26, 168.19.

*4.1.4.14. 4-(2-Amino-2-ethoxycarbonyl-ethyl)-benzoic acid ethyl ester hydrochloride (**15d**).* The product was obtained in 81% yield. TLC (AcOEt/MeOH 1:1) - Rf: 0.80; m.p. 146 °C; ^{1}H-NMR (DMSO-d_6) δ 1.02 (t, 3H, $-CH_3$); 1.27 (t, 3H, $-CH_3$); 3.25 (m, 2H, $-C\underline{H}_2-CH<$); 4.04 (q, 2H, $-CH_2-O-$); 4.26 (m, 3H, –CH< and-CH_2–O–); 7.40 (d, 2H, Ar); 7.87(d, 2H, Ar); 8.89 (b, 3H, $-NH_3^+$); ^{13}C-NMR (DMSO-d_6) δ 13.52, 13.93, 35.48, 52.73, 60.51, 61.44, 128.53, 129.02, 129.65, 140.30, 165.34, 168.37.

*4.1.4.15. 2-Amino-3-(2-nitro-phenyl)-propionic acid ethyl ester hydrochloride (**16d**).* The product was obtained in 94% yield. TLC (AcOEt/MeOH 1:1) - Rf: 0.79; m.p. 215 °C; ^{1}H-NMR (DMSO-d_6) δ 0.95 (t, 3H, $-CH_3$); 3.48 (m, 2H, $-C\underline{H}_2-CH<$); 3.96 (q, 2H, $-CH_2-O-$); 4.19 (m, 1H, –CH<); 7.57–7.74 (m, 3H, Ar); 8.04 (d, 1H, Ar); 9.02 (b, 3H, $-NH_3^+$); ^{13}C-NMR (DMSO-d_6) δ 13.29, 32.58, 52.38, 61.46, 124.63, 128.77, 129.94, 132.96, 133.69, 148.70, 168.21.

*4.1.4.16. 2-Amino-3-(4-nitro-phenyl)-propionic acid ethyl ester hydrochloride (**17d**).* The product was obtained in 95% yield. TLC (AcOEt/MeOH 1:1) - Rf: 0.75; m.p. 173 °C; ^{1}H-NMR (DMSO-d_6) δ 1.07 (t, 3H, $-CH_3$); 3.42 (m, 2H, $-C\underline{H}_2-CH<$); 4.09 (m, 2H, $-CH_2-O-$); 4.31 (m, 1H, –CH<); 7.59 (d, 2H, Ar); 8.16 (d, 2H, Ar); 8.96 (b, 3H, $-NH_3^+$); ^{13}C-NMR (DMSO-d_6) δ 13.59, 35.15, 52.51, 61.55, 123.27, 130.81, 143.10, 146.51, 168.31.

*4.1.4.17. 2-Amino-3-pyridin-3-yl-propionic acid ethyl ester hydrochloride (**18d**).* The hygroscopic product was obtained in 90% yield. TLC (AcOEt/MeOH 1:1) - Rf: 0.44; ^{1}H-NMR (DMSO-d_6) δ 1.12 (t, 3H, $-CH_3$); 3.43 (m, 2H, $-C\underline{H}_2-CH<$); 4.13 (m, 2H, $-CH_2-O-$); 4.45 (m, 1H, –CH<); 8.02 (t, 1H, Ar); 8.55 (d, 1H, Ar); 8.85 (d, 1H, Ar); 8.95 (m, 4H, Ar and

–NH_3^+); ^{13}C-NMR (DMSO-d_6) δ 13.71, 31.91, 51.96, 61.85, 126.70, 135.19, 140.08, 142.26, 146.72, 167.94.

*4.1.4.18. 2-Amino-3-cyclohexyl-propionic acid ethyl ester hydrochloride (**19d**).* The product was obtained in 82% yield. TLC (AcOEt/MeOH 1:1) - Rf: 0.78; m.p. 162 °C; ^{1}H-NMR (DMSO-d_6) δ 0.85 (m, 2H, cyclohexyl); 1.15–1.26 (m, 6H, cyclohexyl and –CH_3); 1.45–1.75 (m, 8H, cyclohexyl and –$C\underline{H}_2$–C_6H_{11}); 3.89 (m, 1H, >$C\underline{H}$–CH_2–C_6H_{11}); 4.18 (m, 2H, –CH_2–O–); 8.75 (b, 3H, –NH_3^+); ^{13}C-NMR (DMSO-d_6) δ 13.75, 25.20, 25.32, 25.62, 32.02, 32.11, 32.47, 37.39, 49.63, 61.35, 169.21.

*4.1.5. General procedure for the synthesis of anthranoyl derivatives **1e–19e***

A suspension of 20.0 mmol of the aminoacid ethyl ester hydrochloride (**1d–19d**) in 150 mL of ethyl acetate was treated with triethylamine (2.78 mL, 20.0 mmol) followed by 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 M NaOH (3 × 50 mL), water (1 × 50 mL) and brine, dried over anhydrous sodium sulfate and concentrated in vacuum. The residue was purified as described to yield the titled compounds.

*4.1.5.1. 2-(2-Amino-benzoylamino)-3-o-tolyl-propionic acid ethyl ester (**1e**).* Crystallization from AcOEt/n-hexane afforded the titled compound in 47% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.61; m.p. 70 °C; ^{1}H-NMR ($CDCl_3$) δ 1.20 (t, 3H, –CH_2–$C\underline{H}_3$); 2.38 (s, 3H, –CH_3); 3.21 (m, 2H, –$C\underline{H}_2$–CH<); 4.16 (q, 2H, –CH_2–O–); 4.98 (m, 1H, –CH<); 5.35 (b, 2H, –NH_2); 6.63 (m, 3H, –NH– and Ar); 7.13–7.31 (m, 6H, Ar); ^{13}C-NMR ($CDCl_3$) δ 13.90, 19.31, 35.72, 52.48, 61.39, 115.17, 116.45, 117.12, 125.86, 127.04, 127.28, 129.76, 130.45, 132.41, 134.37, 136.64, 148.69, 168.65, 172.17.

*4.1.5.2. 2-(2-Amino-benzoylamino)-3-p-tolyl-propionic acid ethyl ester (**2e**).* Purification by flash chromatography (AcOEt/n-hexane 1:1) afforded the titled compound in 55% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.69; m.p. 44 °C; ^{1}H-NMR ($CDCl_3$) δ 1.29 (t, 3H, –CH_2–$C\underline{H}_3$); 2.32 (s, 3H, –CH_3); 3.20 (m, 2H, –$C\underline{H}_2$–CH<); 4.21 (q, 2H, –CH_2–O–); 4.98 (m, 1H, –CH<); 5.40 (b, 2H, –NH_2); 6.52 (d, 1H, –NH–); 6.67 (m, 2H, Ar); 7.08 (m, 4H, Ar); 7.25 (m, 2H, Ar); ^{13}C-NMR ($CDCl_3$) δ 14.29, 21.19, 37.61, 53.43, 61.65, 115.48, 116.72, 117.33, 127.49, 129.33, 129.56, 132.61, 132.87, 136.76, 148.87, 168.67, 171.81.

*4.1.5.3. 2-(2-Amino-benzoylamino)-3-(4-ethyl-phenyl)-propionic acid ethyl ester (**3e**).* Crystallization from AcOEt/n-hexane afforded the titled compound in 50% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.70; m.p. 67–68 °C; ^{1}H-NMR ($CDCl_3$) δ 1.20 (t, 3H, –CH_2–$C\underline{H}_3$); 1.25 (t, 3H, –O–CH_2–$C\underline{H}_3$); 2.60 (q, 2H, –$C\underline{H}_2$–CH_3); 3.18 (m, 2H, –$C\underline{H}_2$–CH<); 4.19 (q, 2H, –CH_2–O–); 4.97 (m, 1H, –CH<); 5.47 (b, 2H, –NH_2); 6.51 (d, 1H, –NH–); 6.62 (m, 2H, Ar); 7.08 (m, 4H, Ar); 7.23 (m, 2H, Ar); ^{13}C-NMR ($CDCl_3$) δ 14.32, 15.71, 28.61, 37.68, 53.48, 61.66, 115.45, 116.67, 117.29, 127.49, 128.14, 129.37, 132.58, 133.11, 143.09, 148.86, 168.65, 171.80.

*4.1.5.4. 2-(2-Amino-benzoylamino)-3-(4-tert-butyl-phenyl)-propionic acid ethyl ester (**4e**).* Purification by flash chromatography (AcOEt/n-hexane 1:1) afforded the titled compound in 60% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.69; m.p. 104 °C; ^{1}H-NMR ($CDCl_3$) δ 1.30 (m, 12H, –$C(CH_3)_3$ and –CH_2–$C\underline{H}_3$); 3.20 (m, 2H, –$C\underline{H}_2$–CH<); 4.21 (q, 2H, –CH_2–O–); 4.98 (m, 1H, –CH<); 5.38 (b, 2H, –NH_2); 6.51 (d, 1H, –NH–); 6.65 (m, 2H, Ar); 7.09 (d, 2H, Ar); 7.21–7.33 (m, 4H, Ar); ^{13}C-NMR ($CDCl_3$) δ 14.23, 31.43, 37.55, 53.42, 61.60, 115.55, 116.72, 117.31, 125.58, 127.53, 129.12, 132.60, 132.90, 148.83, 150.03, 168.71, 171.87.

*4.1.5.5. 2-(2-Amino-benzoylamino)-3-biphenyl-4-yl-propionic acid ethyl ester (**5e**).* Crystallization from AcOEt/n-hexane afforded the titled compound in 66% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.65; m.p. 107 °C; ^{1}H-NMR ($CDCl_3$) δ 1.28 (t, 3H, –CH_3); 3.27 (m, 2H, –$C\underline{H}_2$–CH<); 4.22 (q, 2H, –CH_2–O–); 5.04 (m, 1H, –CH<); 5.48 (b, 2H, –NH_2); 6.56–6.68 (m, 3H, –NH– and Ar); 7.20–7.59 (m, 11H, Ar); ^{13}C-NMR ($CDCl_3$) δ 14.36, 37.76, 53.45, 61.79, 115.36, 116.74, 117.34, 127.07, 127.31, 127.48, 128.83, 129.91, 132.67, 135.12, 140.01, 140.72, 148.89, 168.68, 171.72.

*4.1.5.6. 2-(2-Amino-benzoylamino)-3-(2-methoxy-phenyl)-propionic acid ethyl ester (**6e**).* Crystallization from AcOEt/n-hexane afforded the titled compound in 47% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.59; m.p. 103–104 °C; ^{1}H-NMR ($CDCl_3$) δ 1.25 (t, 3H, –CH_2–$C\underline{H}_3$); 3.23 (m, 2H, –$C\underline{H}_2$–CH<); 3.83 (s, 3H, –O–CH_3); 4.18 (q, 2H, –CH_2–O–); 4.81 (m, 1H, –CH<); 5.30 (b, 2H, –NH_2); 6.60 (m, 2H, Ar); 6.89 (m, 2H, Ar); 7.09–7.28 (m, 5H, –NH– and Ar); ^{13}C-NMR ($CDCl_3$) δ 14.01, 32.08, 53.88, 55.26, 61.07, 110.39, 115.24, 116.13, 117.02, 120.84, 124.72, 127.17, 128.48, 131.17, 132.17, 148.71, 157.20, 168.83, 171.84.

*4.1.5.7. 2-(2-Amino-benzoylamino)-3-(3-methoxy-phenyl)-propionic acid ethyl ester (**7e**).* Crystallization from AcOEt/n-hexane afforded the titled compound in 70% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.61; m.p. 74–75 °C; ^{1}H-NMR ($CDCl_3$) δ 1.26 (t, 3H, –CH_2–$C\underline{H}_3$); 3.20 (m, 2H, –$C\underline{H}_2$–CH<); 3.72 (s, 3H, –O–CH_3); 4.19 (q, 2H, –CH_2–O–); 4.99 (m, 1H, –CH<); 5.23 (b, 2H, –NH_2); 6.55–6.80 (m, 6H, –NH– and Ar); 7.14–7.29 (m, 3H, Ar); ^{13}C-NMR ($CDCl_3$) δ 14.33, 38.06, 53.37, 55.23, 61.74, 112.81, 114.92, 115.38, 116.70, 117.30, 121.74, 127.47, 129.61, 132.63, 137.52, 148.83, 159.69, 168.65, 171.68.

*4.1.5.8. 2-(2-Amino-benzoylamino)-3-(4-methoxy-phenyl)-propionic acid ethyl ester (**8e**).* Purification by flash chromatography (AcOEt/n-hexane 1:3) afforded the titled compound in 27% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.62;

m.p. 62 °C; ^{1}H-NMR ($CDCl_3$) δ 1.28 (t, 3H, –CH_2–CH_3); 3.17 (m, 2H, –CH_2–CH<); 3.77 (s, 3H, –O–CH_3); 4.20 (q, 2H, –CH_2–O–); 4.96 (m, 1H, –CH<); 5.50 (b, 2H, –NH_2); 6.63 (m, 3H, –NH– and Ar); 6.82 (d, 2H, Ar); 7.07 (d, 2H, Ar); 7.25 (m, 2H, Ar); ^{13}C-NMR ($CDCl_3$) δ 14.09, 36.98, 53.36, 55.10, 61.44, 113.89, 115.26, 116.49, 117.13, 127.30, 127.84, 130.28, 132.43, 148.75, 158.59, 168.51, 171.68.

*4.1.5.9. 2-(2-Amino-benzoylamino)-3-(4-fluoro-phenyl)-propionic acid ethyl ester (**9e**).* Crystallization from AcOEt/n-hexane afforded the titled compound in 59% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.65; m.p. 87–88 °C; ^{1}H-NMR ($CDCl_3$) δ 1.25 (t, 3H, –CH_3); 3.18 (m, 2H, –CH_2–CH<); 4.18 (q, 2H, –CH_2–O–); 4.98 (m, 1H, –CH<); 5.20 (b, 2H, –NH_2); 6.63 (m, 3H, –NH– and Ar); 6.95–7.28 (m, 6H, Ar); ^{13}C-NMR ($CDCl_3$) δ 14.09, 37.12, 53.29, 61.62, 115.10, 115.21, 115.42, 116.59, 117.23, 127.25, 130.67, 130.77, 130.85, 131.68, 132.58, 148.78, 160.70, 163.14, 168.55, 171.52.

*4.1.5.10. 2-(2-Amino-benzoylamino)-3-(2-chloro-phenyl)-propionic acid ethyl ester (**10e**).* Crystallization from AcOEt/n-hexane afforded the titled compound in 24% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.67; m.p. 99–102 °C; ^{1}H-NMR ($CDCl_3$) δ 1.22 (t, 3H, –CH_3); 3.34 (m, 2H, –CH_2–CH<); 4.17 (q, 2H, –CH_2–O–); 5.01 (m, 1H, –CH<); 5.25 (b, 2H, –NH_2); 6.60–6.71 (m, 3H, –NH– and Ar); 7.13–7.38 (m, 6H, Ar); ^{13}C-NMR ($CDCl_3$) δ 13.91, 35.45, 52.58, 61.56, 115.03, 116.40, 117.08, 126.83, 127.32, 128.41, 129.49, 131.29, 132.41, 134.23, 134.29, 148.72, 168.71, 171.70.

*4.1.5.11. 2-(2-Amino-benzoylamino)-3-(3-chloro-phenyl)-propionic acid ethyl ester (**11e**).* Trituration with diethyl ether/n-hexane afforded the titled compound in 19% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.70; m.p. 55 °C; ^{1}H-NMR ($CDCl_3$) δ 1.26 (t, 3H, –CH_3); 3.19 (m, 2H, –CH_2–CH<); 4.19 (q, 2H, –CH_2–O–); 4.98 (m, 1H, –CH<); 5.25 (b, 2H, –NH_2); 6.58–6.67 (m, 3H, –NH– and Ar); 7.02–7.30 (m, 6H, Ar); ^{13}C-NMR ($CDCl_3$) δ 14.27, 37.75, 53.32, 61.88, 115.20, 116.74, 117.37, 127.34, 127.49, 127.68, 129.63, 129.86, 132.75, 134.31, 138.28, 148.95, 168.78, 171.53.

*4.1.5.12. 2-(2-Amino-benzoylamino)-3-(4-chloro-phenyl)-propionic acid ethyl ester (**12e**).* Crystallization from AcOEt/n-hexane afforded the titled compound in 53% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.66; m.p. 93 °C; ^{1}H-NMR ($CDCl_3$) δ 1.27 (t, 3H, –CH_3); 3.20 (m, 2H, –CH_2–CH<); 4.18 (q, 2H, –CH_2–O–); 4.98 (m, 1H, –CH<); 5.28 (b, 2H, –NH_2); 6.64 (m, 3H, –NH– and Ar); 7.06–7.27 (m, 6H, Ar); ^{13}C-NMR ($CDCl_3$) δ 14.09, 37.25, 53.15, 61.66, 115.00, 116.59, 117.22, 127.25, 128.57, 130.66, 132.59, 132.89, 134.50, 148.78, 168.55, 171.40.

*4.1.5.13. 2-(2-Amino-benzoylamino)-3-(3,5-dichloro-phenyl)-propionic acid ethyl ester (**13e**).* Crystallization from AcOEt/n-hexane afforded the titled compound in 39% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.59; m.p. 105–106 °C; ^{1}H-NMR ($CDCl_3$) δ 1.26 (t, 3H, –CH_3); 3.15 (m, 2H, –CH_2–CH<); 4.20 (q, 2H, –CH_2–O–); 4.95 (m, 1H, –CH<); 5.48 (b, 2H, –NH_2); 6.64 (m, 3H, –NH– and Ar); 7.05 (s, 2H, Ar); 7.17–7.31 (m, 3H, Ar); ^{13}C-NMR ($CDCl_3$) δ 14.32, 37.67, 53.21, 62.09, 115.01, 116.81, 117.39, 127.38, 127.40, 128.02, 132.85, 134.93, 139.62, 148.92, 168.74, 171.18.

*4.1.5.14. 2-(2-Amino-benzoylamino)-3-(2,6-dichloro-phenyl)-propionic acid ethyl ester (**14e**).* Crystallization from AcOEt/n-hexane afforded the titled compound in 34% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.76; m.p. 105 °C; ^{1}H-NMR ($CDCl_3$) δ 1.09 (t, 3H, –CH_3); 3.47 (m, 2H, –CH_2–CH<); 4.05 (q, 2H, –CH_2–O–); 4.75 (m, 1H, –CH<); 5.95 (b, 2H, –NH_2); 6.65 (m, 3H, –NH– and Ar); 7.10–7.50 (m, 5H, Ar); ^{13}C-NMR ($CDCl_3$) δ 13.45, 32.18, 50.81, 60.49, 113.78, 114.56, 116.12, 127.71, 127.94, 128.30, 131.59, 133.18, 135.29, 149.09, 168.74, 170.86.

*4.1.5.15. 4-[2-(2-Amino-benzoylamino)-2-ethoxycarbonyl-ethyl]-benzoic acid ethyl ester (**15e**).* Crystallization from MeOH afforded the titled compound in 37% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.38; m.p. 106–107 °C; ^{1}H-NMR ($CDCl_3$) δ 1.24 (t, 3H, –CH_3); 1.36 (t, 3H, –CH_3); 3.26 (m, 2H, –CH_2–CH<); 4.17 (q, 2H, –CH_2–O–); 4.33 (q, 2H, –CH_2–O–); 5.01 (m, 1H, –CH<); 5.35 (b, 2H, –NH_2); 6.62 (m, 3H, –NH– and Ar); 7.24 (m, 4H, Ar); 7.95 (d, 2H, Ar); ^{13}C-NMR ($CDCl_3$) δ 14.08, 14.24, 37.87, 53.05, 60.86, 61.68, 114.95, 116.55, 117.19, 127.25, 129.22, 129.32, 129.65, 132.58, 141.33, 148.79, 166.30, 168.57, 171.31.

*4.1.5.16. 2-(2-Amino-benzoylamino)-3-(2-nitro-phenyl)-propionic acid ethyl ester (**16e**).* Crystallization from AcOEt/n-hexane afforded the titled compound in 49% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.55; m.p. 102–103 °C; ^{1}H-NMR ($CDCl_3$) δ 1.22 (t, 3H, –CH_3); 3.48 (m, 2H, –CH_2–CH<); 4.16 (q, 2H, –CH_2–O–); 5.04 (m, 1H, –CH<); 5.45 (b, 2H, –NH_2); 6.61 (m, 2H, Ar); 6.93 (d, 1H, –NH–); 7.16 (t, 1H, Ar); 7.30–7.56 (m, 4H, Ar); 7.91 (d, 1H, Ar); ^{13}C-NMR ($CDCl_3$) δ 13.92, 34.70, 53.16, 61.81, 114.68, 116.45, 117.12, 124.83, 127.24, 128.10, 131.71, 132.54, 132.68, 133.07, 148.81, 149.59, 168.79, 171.36.

*4.1.5.17. 2-(2-Amino-benzoylamino)-3-(4-nitro-phenyl)-propionic acid ethyl ester (**17e**).* Crystallization from AcOEt/n-hexane afforded the titled compound in 28% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.53; m.p. 84–86 °C; ^{1}H-NMR ($CDCl_3$) δ 1.27 (t, 3H, –CH_3); 3.33 (m, 2H, –CH_2–CH<); 4.21 (q, 2H, –CH_2–O–); 5.03 (m, 1H, –CH<); 5.15 (b, 2H, –NH_2); 6.65 (m, 3H, –NH– and Ar); 7.17–7.35 (m, 4H, Ar); 8.12 (d, 2H, Ar); ^{13}C-NMR ($CDCl_3$) δ 14.09, 37.81, 52.97, 61.93, 114.65, 116.59, 117.29, 123.54, 127.16, 130.23, 132.76, 144.01, 147.00, 148.87, 168.64, 171.07.

*4.1.5.18. 2-(2-Amino-benzoylamino)-3-pyridin-3-yl-propionic acid ethyl ester (**18e**).* Purification by flash chromatography (AcOEt/MeOH 95:5) afforded the titled compound as

oil in 35% yield. TLC (AcOEt/MeOH 2:1) - Rf: 0.79; ^{1}H-NMR ($CDCl_3$) δ 1.22 (t, 3H, –CH_3); 3.20 (m, 2H, –C$\underline{H}_2$–CH<); 4.16 (q, 2H, –CH_2–O–); 4.80 (b, 2H, –NH_2); 4.96 (m, 1H, –CH<); 6.64 (m, 3H, –NH– and Ar); 7.21 (m, 3H, Ar); 7.52 (d, 1H, Ar); 8.44 (m, 2H, Ar); ^{13}C-NMR ($CDCl_3$) δ 14.29, 35.36, 53.21, 62.03, 114.97, 116.73, 117.36, 123.49, 127.45, 131.96, 132.79, 137.06, 148.41, 148.93, 150.40, 168.81, 171.31.

*4.1.5.19. 2-(2-Amino-benzoylamino)-3-cyclohexyl-propionic acid ethyl ester (**19e**).* Purification by flash chromatography (AcOEt/n-hexane 1:1) afforded the titled compound as oil in 30% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.75; ^{1}H-NMR ($CDCl_3$) δ 0.84–1.00 (m, 3H, -cyclohexyl); 1.14–1.40 (m, 7H, cyclohexyl and –CH_3); 1.61–1.82 (m, 6H, cyclohexyl and –C$\underline{H}_2$–C_6H_{11}); 4.20 (q, 2H, –CH_2–O–); 4.78 (m, 1H, >C$\underline{H}$–CH_2–C_6H_{11}); 5.15 (b, 2H, –NH_2); 6.47 (d, 1H, –NH–); 6.65 (m, 2H, Ar); 7.21 (t, 1H, Ar); 7.39 (d, 1H, Ar); ^{13}C-NMR ($CDCl_3$) δ 14.29, 26.13, 26.26, 26.47, 32.78, 33.57, 34.34, 40.42, 50.37, 61.45, 115.63, 116.66, 117.32, 127.51, 132.56, 148.84, 168.95, 173.49.

*4.1.6. General procedure for the synthesis of derivatives **1f–19f***

To a suspension of 0.81 g (5.00 mmol) of indol-2-carboxylic acid in 20 mL of acetyl chloride cooled in an ice-bath were added portionwise, over a period of 0.5 h, 1.04 g (5.00 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 4.00 mmol of the derivatives **1e–19e** in 4 mL of pyridine. After the addition was completed, the reaction mixture was stirred at room temperature overnight. Then 150 mL of CH_2Cl_2 were added and the organic layer was washed twice with 40 mL of N HCl, H_2O, 0.1 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.

Compound **18f** precipitated at the interphase after the addition of N HCl and was filtered.

*4.1.6.1. 2-{2-[(1H-Indole-2-carbonyl)-amino]-benzoylamino}-3-o-tolyl-propionic acid ethyl ester (**1f**).* Trituration with hot MeOH afforded the titled compound in 84% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.72; m.p. 217–218 °C; ^{1}H-NMR (DMSO-d_6) δ 1.15 (t, 3H, –CH_2–C$\underline{H}_3$); 2.37 (s, 3H, –CH_3); 3.21 (m, 2H, –C$\underline{H}_2$–CH<); 4.13 (q, 2H, –CH_2–O–); 4.77 (m, 1H, –CH<); 7.00 (s, 1H, Ar); 7.07–7.40 (m, 7H, Ar); 7.51 (d, 1H, Ar); 7.61 (t, 1H, Ar); 7.72 (d, 1H, Ar); 7.84 (d, 1H, Ar); 8.66 (d, 1H, Ar); 9.36 (d, 1H, –N$\underline{H}$–CH<); 11.97 (s, 1H, –NH–); 12.16 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 13.96, 18.90, 33.63, 52.97, 60.81, 102.65, 112.53, 119.38, 119.95, 120.22, 121.75, 122.56, 124.07, 125.64, 126.66, 126.96, 128.50, 129.64, 130.10, 131.53, 132.64, 135.60, 136.08, 137.16, 139.17, 159.14, 168.98, 171.37.

*4.1.6.2. 2-{2-[(1H-Indole-2-carbonyl)-amino]-benzoylamino}-3-p-tolyl-propionic acid ethyl ester (**2f**).* Trituration with hot MeOH afforded the titled compound in 16% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.71; m.p. 186 °C; ^{1}H-NMR (DMSO-d_6) δ 1.17 (t, 3H, –CH_2–C$\underline{H}_3$); 2.15 (s, 3H, –CH_3); 3.14 (m, 2H, –C$\underline{H}_2$–CH<); 4.14 (q, 2H, –CH_2–O–); 4.75 (m, 1H, –CH<); 6.95 (s, 1H, Ar); 7.08 (m, 3H, Ar); 7.24 (m, 4H, Ar); 7.49 (d, 1H, Ar); 7.61 (t, 1H, Ar); 7.72 (d, 1H, Ar); 7.81 (d, 1H, Ar); 8.63 (d, 1H, Ar); 9.28 (d, 1H, –N$\underline{H}$–CH<); 11.94 (s, 1H, –NH–); 12.12 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 13.85, 20.37, 35.56, 54.28, 60.61, 102.40, 112.32, 119.18, 119.72, 120.03, 121.58, 122.39, 123.90, 126.75, 128.26, 128.61, 128.77, 131.30, 132.45, 134.15, 135.34, 136.94, 138.93, 158.90, 168.68, 171.05.

*4.1.6.3. 3-(4-Ethyl-phenyl)-2-{2-[(1H-indole-2-carbonyl)-amino]-benzoylamino}-propionic acid ethyl ester (**3f**).* Trituration with hot MeOH afforded the titled compound in 59% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.60; m.p. 183 °C; ^{1}H-NMR (DMSO-d_6) δ 0.99–1.14 (m, 6H, –CH_3); 2.45 (q, 2H, –C$\underline{H}_2$–CH_3); 3.11 (m, 2H, –C$\underline{H}_2$–CH<); 4.09 (q, 2H, –CH_2–O–); 4.69 (m, 1H, –CH<); 6.92 (s, 1H, Ar); 7.07 (m, 3H, Ar); 7.20 (m, 4H, Ar); 7.43 (d, 1H, Ar); 7.56 (t, 1H, Ar); 7.67 (d, 1H, Ar); 7.76 (d, 1H, Ar); 8.58 (d, 1H, Ar); 9.23 (d, 1H, –N$\underline{H}$–CH<); 11.88 (s, 1H, –NH–); 12.08 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 14.79, 16.24, 28.44, 36.45, 55.21, 61.53, 103.30, 113.21, 120.05, 120.61, 120.95, 122.47, 123.32, 124.82, 127.61, 128.30, 129.16, 129.70, 132.16, 133.37, 135.27, 137.79, 139.76, 142.58, 159.75, 169.58, 171.95.

*4.1.6.4. 3-(4-tert-Butyl-phenyl)-2-{2-[(1H-indole-2-carbonyl)-amino]-benzoylamino}-propionic acid ethyl ester (**4f**).* Trituration with hot MeOH afforded the titled compound in 67% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.76; m.p. 212 °C; ^{1}H-NMR (DMSO-d_6) δ 1.18 (m, 12H, –$C(CH_3)_3$ and-CH_2–C$\underline{H}_3$); 3.18 (m, 2H, –C$\underline{H}_2$–CH<); 4.12 (q, 2H, –CH_2–O–); 4.75 (m, 1H, –CH<); 7.00 (s, 1H, Ar); 7.10 (t, 1H, Ar); 7.19–7.27 (m, 6H, Ar); 7.49 (d, 1H, Ar); 7.61 (t, 1H, Ar); 7.72 (d, 1H, Ar); 7.82 (d, 1H, Ar); 8.63 (d, 1H, Ar); 9.30 (d, 1H, –N$\underline{H}$–CH<); 11.94 (s, 1H, –NH–); 12.17 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 13.80, 30.88, 33.85, 35.34, 54.23, 60.55, 102.40, 112.32, 119.19, 119.74, 120.02, 121.58, 122.38, 123.89, 124.78, 126.75, 128.31, 128.50, 131.32, 132.46, 134.15, 136.95, 138.95, 148.60, 158.91, 168.80, 171.12.

*4.1.6.5. 3-Biphenyl-4-yl-2-{2-[(1H-indole-2-carbonyl)-amino]-benzoylamino}-propionic acid ethyl ester (**5f**).* Trituration with hot MeOH afforded the titled compound in 45% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.53; m.p. 224–225 °C; ^{1}H-NMR (DMSO-d_6) δ 1.12 (t, 3H, –CH_3); 3.20 (m, 2H, –C$\underline{H}_2$–CH<); 4.11 (q, 2H, –CH_2–O–); 4.78 (m, 1H, –CH<); 6.95 (s, 1H, Ar); 7.05 (t, 1H, Ar); 7.15–760 (m, 13H, Ar); 7.67 (d, 1H, Ar); 7.78 (d, 1H, Ar); 8.57 (d, 1H, Ar); 9.28 (d, 1H, –N$\underline{H}$–CH<); 11.88 (s, 1H, –NH–); 12.10 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 14.82, 36.41, 55.02, 61.60,

103.29, 113.22, 120.08, 120.65, 120.95, 122.49, 123.34, 124.82, 127.13, 127.20, 127.63, 127.92, 129.19, 129.51, 130.39, 132.16, 133.39, 137.44, 137.82, 139.07, 139.80, 140.45, 159.76, 169.65, 171.90.

*4.1.6.6. 2-{2-[(1H-Indole-2-carbonyl)-amino]-benzoylamino}-3-(2-methoxy-phenyl)-propionic acid ethyl ester (**6f**).* Trituration with hot MeOH afforded the titled compound in 65% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.68; m.p. 197 °C; ^{1}H-NMR (DMSO-d_6) δ 1.28 (t, 3H, –CH_2–$C\underline{H}_3$); 3.33 (m, 2H, –$C\underline{H}_2$–CH<); 3.98 (s, 3H, –O–CH_3); 4.26 (q, 2H, –CH_2–O–); 4.96 (m, 1H, –CH<); 6.98 (t, 1H, Ar); 7.14 (m, 2H, Ar); 7.21–7.37 (m, 5H, Ar); 7.65 (d, 1H, Ar); 7.75 (t, 1H, Ar); 7.87 (d, 1H, Ar); 7.94 (d, 1H, Ar); 8.80 (d, 1H, Ar); 9.38 (d, 1H, –N$\underline{H}$–CH<); 11.95 (s, 1H, –NH–); 12.10 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 13.97, 31.40, 52.60, 55.33, 60.64, 102.59, 110.54, 112.50, 119.30, 119.87, 120.02, 120.21, 121.74, 122.52, 124.07, 125.02, 126.94, 128.16, 128.43, 130.76, 131.53, 132.60, 137.12, 139.15, 157.29, 159.09, 168.88, 171.38.

*4.1.6.7. 2-{2-[(1H-Indole-2-carbonyl)-amino]-benzoylamino}-3-(3-methoxy-phenyl)-propionic acid ethyl ester (**7f**).* Trituration with hot MeOH afforded the titled compound in 74% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.64; m.p. 178–179 °C; ^{1}H-NMR (DMSO-d_6) δ 1.17 (t, 3H, –CH_2–$C\underline{H}_3$); 3.19 (m, 2H, –$C\underline{H}_2$–CH<); 3.67 (s, 3H, –O–CH_3); 4.15 (q, 2H, –CH_2–O–); 4.79 (m, 1H, –CH<); 6.70 (d, 1H, Ar); 6.94 (m, 3H, Ar); 7.22 (m, 4H, Ar); 7.50 (d, 1H, Ar); 7.61 (t, 1H, Ar); 7.71 (d, 1H, Ar); 7.82 (d, 1H, Ar); 8.66 (d, 1H, Ar); 9.30 (d, 1H, –N$\underline{H}$–CH<); 11.96 (s, 1H, –NH–); 12.19 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 14.01, 36.09, 54.30, 54.80, 60.80, 102.61, 112.04, 112.52, 114.70, 119.29, 119.91, 120.22, 121.32, 121.76, 122.53, 124.08, 126.95, 128.45, 129.20, 131.53, 132.66, 137.15, 139.00, 139.21, 159.14, 168.90, 171.19.

*4.1.6.8. 2-{2-[(1H-Indole-2-carbonyl)-amino]-benzoylamino}-3-(4-methoxy-phenyl)-propionic acid ethyl ester (**8f**).* Trituration with hot MeOH afforded the titled compound in 79% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.69; m.p. 189 °C; ^{1}H-NMR (DMSO-d_6) δ 1.16 (t, 3H, –CH_2–$C\underline{H}_3$); 3.13 (m, 2H, –$C\underline{H}_2$–CH<); 3.62 (s, 3H, –O–CH_3); 4.13 (q, 2H, –CH_2–O–); 4.74 (m, 1H, –CH<); 6.83 (m, 2H, Ar); 6.98 (s, 1H, Ar); 7.10 (t, 1H, Ar); 7.25 (m, 4H, Ar); 7.49 (d, 1H, Ar); 7.61 (t, 1H, Ar); 7.72 (d, 1H, Ar); 7.82 (d, 1H, Ar); 8.64 (d, 1H, Ar); 9.26 (d, 1H, –N$\underline{H}$–CH<); 11.94 (s, 1H, –NH–); 12.16 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 13.98, 35.24, 54.58, 54.79, 60.71, 102.54, 112.46, 113.61, 119.35, 119.89, 120.17, 121.73, 122.54, 124.05, 126.92, 128.44, 129.19, 130.05, 131.49, 132.60, 137.11, 139.11, 157.92, 159.07, 168.88, 171.24.

*4.1.6.9. 3-(4-Fluoro-phenyl)-2-{2-[(1H-indole-2-carbonyl)-amino]-benzoylamino}-propionic acid ethyl ester (**9f**).* Trituration with hot MeOH afforded the titled compound in 68% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.63; m.p. 185 °C; ^{1}H-NMR (DMSO-d_6) δ 1.15 (t, 3H, –CH_3); 3.19 (m, 2H, –$C\underline{H}_2$–CH<); 4.14 (q, 2H, –CH_2–O–); 4.78 (m, 1H, –CH<); 6.98 (s, 1H, Ar); 7.06–7.52 (m, 8H, Ar); 7.61 (t, 1H, Ar); 7.71 (d, 1H, Ar); 7.81 (d, 1H, Ar); 8.66 (d, 1H, Ar); 9.31 (d, 1H, –N$\underline{H}$–CH<); 11.96 (s, 1H, –NH–); 12.17 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 13.97, 35.24, 54.34, 60.78, 102.57, 112.50, 114.80, 115.01, 119.30, 119.93, 120.20, 121.73, 122.56, 124.07, 126.95, 128.43, 130.92, 131.00, 131.52, 132.64, 133.58, 137.15, 139.17, 159.12, 159.85, 162.26, 168.93, 171.09.

*4.1.6.10. 3-(2-Chloro-phenyl)-2-{2-[(1H-indole-2-carbonyl)-amino]-benzoylamino}-propionic acid ethyl ester (**10f**).* Trituration with hot MeOH afforded the titled compound in 71% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.69; m.p. 207–208 °C; ^{1}H-NMR (DMSO-d_6) δ 1.16 (t, 3H, –CH_3); 3.35 (m, 2H, –$C\underline{H}_2$–CH<); 4.15 (q, 2H, –CH_2–O–); 4.87 (m, 1H, –CH<); 6.98 (s, 1H, Ar); 7.07–7.53 (m, 8H, Ar); 7.62 (t, 1H, Ar); 7.73 (d, 1H, Ar); 7.82 (d, 1H, Ar); 8.65 (d, 1H, Ar); 9.38 (d, 1H, –N$\underline{H}$–CH<); 11.95 (s, 1H, –NH–); 12.10 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 13.95, 34.01, 52.32, 60.94, 102.64, 112.50, 119.29, 119.97, 120.21, 121.76, 122.58, 124.08, 126.94, 126.99, 128.41, 128.64, 129.22, 131.48, 131.76, 132.67, 133.33, 134.90, 137.13, 139.12, 159.10, 168.94, 170.87.

*4.1.6.11. 3-(3-Chloro-phenyl)-2-{2-[(1H-indole-2-carbonyl)-amino]-benzoylamino}-propionic acid ethyl ester (**11f**).* Trituration with hot MeOH afforded the titled compound in 64% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.68; m.p. 207–208 °C; ^{1}H-NMR (DMSO-d_6) δ 1.15 (t, 3H, –CH_3); 3.23 (m, 2H, –$C\underline{H}_2$–CH<); 4.14 (q, 2H, –CH_2–O–); 4.80 (m, 1H, –CH<); 6.97 (s, 1H, Ar); 7.06–7.52 (m, 8H, Ar); 7.61 (t, 1H, Ar); 7.71 (d, 1H, Ar); 7.79 (d, 1H, Ar); 8.66 (d, 1H, Ar); 9.32 (d, 1H, –N$\underline{H}$–CH<); 11.95 (s, 1H, –NH–); 12.12 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 13.97, 35.62, 53.94, 60.83, 102.58, 112.49, 119.30, 119.94, 120.18, 121.73, 122.54, 124.05, 126.50, 126.94, 127.83, 128.36, 129.06, 129.93, 131.50, 132.65, 132.80, 137.13, 139.15, 140.00, 159.10, 168.87, 170.91.

*4.1.6.12. 3-(4-Chloro-phenyl)-2-{2-[(1H-indole-2-carbonyl)-amino]-benzoylamino}-propionic acid ethyl ester (**12f**).* Trituration with hot MeOH afforded the titled compound in 72% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.65; m.p. 205 °C; ^{1}H-NMR (DMSO-d_6) δ 1.15 (t, 3H, –CH_3); 3.20 (m, 2H, –$C\underline{H}_2$–CH<); 4.13 (q, 2H, –CH_2–O–); 4.79 (m, 1H, –CH<); 6.98 (s, 1H, Ar); 7.06–7.40 (m, 7H, Ar); 7.49 (d, 1H, Ar); 7.61 (t, 1H, Ar); 7.71 (d, 1H, Ar); 7.80 (d, 1H, Ar); 8.65 (d, 1H, Ar); 9.31 (d, 1H, –N$\underline{H}$–CH<); 11.95 (s, 1H, –NH–); 12.16 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 13.98, 35.34, 54.11, 60.84, 102.55, 112.50, 119.22, 119.93, 120.19, 121.76, 122.57, 124.07, 126.95, 128.14, 128.42, 131.00, 131.29, 131.51, 132.69, 136.48, 137.14, 139.18, 159.11, 168.93, 171.01.

*4.1.6.13. 3-(3,5-Dichloro-phenyl)-2-{2-[(1H-indole-2-carbonyl)-amino]-benzoylamino}-propionic acid ethyl ester (**13f**).* Trituration with hot MeOH followed by flash chromatography on silica gel (CH_2Cl_2) afforded the titled compound in 38% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.59; m.p. 206–207 °C; ^{1}H-NMR (DMSO-d_6) δ 1.12 (t, 3H, –CH_3); 3.16 (m, 2H, –C<u>H</u>$_2$–CH<); 4.10 (q, 2H, –CH_2–O–); 4.80 (m, 1H, –CH<); 6.88 (s, 1H, Ar); 7.05 (t, 1H, Ar); 7.15–7.28 (m, 3H, Ar); 7.38 (s, 2H, Ar); –7.44 (d, 1H, Ar); 7.57 (t, 1H, Ar); 7.66 (d, 1H, Ar); 7.72 (d, 1H, Ar); 8.60 (d, 1H, Ar); 9.24 (d, 1H, –N<u>H</u>–CH<); 11.89 (s, 1H, –NH–); 11.97 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 14.80, 36.16, 54.43, 61.70, 103.30, 113.23, 119.94, 120.65, 120.90, 122.50, 123.28, 124.78, 126.94, 127.64, 128.81, 129.04, 132.20, 133.44, 134.36, 137.84, 139.87, 142.51, 159.77, 169.54, 171.38.

*4.1.6.14. 3-(2,6-Dichloro-phenyl)-2-{2-[(1H-indole-2-carbonyl)-amino]-benzoylamino}-propionic acid ethyl ester (**14f**).* Trituration with hot MeOH afforded the titled compound in 74% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.69; m.p. 223 °C; ^{1}H-NMR (DMSO-d_6) δ 1.07 (t, 3H, –CH_3); 3.57 (m, 2H, –C<u>H</u>$_2$–CH<); 4.09 (q, 2H, –CH_2–O–); 4.74 (m, 1H, –CH<); 6.98 (s, 1H, Ar); 7.06–7.33 (m, 4H, Ar); 7.38–7.52 (m, 3H, Ar); 7.61 (t, 1H, Ar); 7.71 (d, 1H, Ar); 7.85 (d, 1H, Ar); 8.65 (d, 1H, Ar); 9.47 (d, 1H, –N<u>H</u>–CH<); 11.96 (s, 1H, –NH–); 12.13 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 13.76, 31.84, 51.60, 61.01, 102.60, 112.50, 119.39, 120.02, 120.20, 121.73, 122.58, 124.07, 126.92, 128.41, 128.50, 129.39, 131.48, 132.62, 133.30, 135.29, 137.13, 139.13, 159.11, 168.91, 170.24.

*4.1.6.15. 4-(2-Ethoxycarbonyl-2-{2-[(1H-indole-2-carbonyl)-amino]-benzoylamino}-ethyl)-benzoic acid ethyl ester (**15f**).* Trituration with hot MeOH afforded the titled compound in 42% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.59; m.p. 187–188 °C; ^{1}H-NMR (DMSO-d_6) δ 1.18 (m, 6H, –CH_3); 3.26 (m, 2H, –C<u>H</u>$_2$–CH<); 4.14 (m, 4H, –CH_2–O–); 4.86 (m, 1H, –CH<); 6.91 (s, 1H, Ar); 7.09 (t, 1H, Ar); 7.23 (m, 2H, Ar); 7.46–7.88 (m, 8H, Ar); 8.64 (d, 1H, Ar); 9.34 (d, 1H, –N<u>H</u>–CH<); 11.92 (s, 1H, –NH–); 12.07 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 14.01, 36.07, 53.85, 60.46, 60.91, 102.54, 112.48, 119.23, 119.86, 120.16, 121.75, 122.57, 124.06, 126.93, 128.28, 128.41, 129.03, 129.49, 131.45, 132.68, 137.13, 139.15, 143.17, 159.07, 165.43, 168.87, 170.92.

*4.1.6.16. 2-{2-[(1H-Indole-2-carbonyl)-amino]-benzoylamino}-3-(2-nitro-phenyl)-propionic acid ethyl ester (**16f**).* Trituration with hot MeOH afforded the titled compound in 67% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.54; m.p. 212 °C; ^{1}H-NMR (DMSO-d_6) δ 1.14 (t, 3H, –CH_3); 3.25–3.74 (m, 2H, –C<u>H</u>$_2$–CH<); 4.13 (q, 2H, –CH_2–O–); 4.98 (m, 1H, –CH<); 6.94 (s, 1H, Ar); 7.07–7.41 (m, 4H, Ar); 7.49–7.63 (m, 4H, Ar); 7.74 (m, 2H, Ar); 7.93 (d, 1H, Ar); 8.61 (d, 1H, Ar); 9.37 (d, 1H, –N<u>H</u>–CH<); 11.91 (s, 1H, –NH–); 11.98 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 13.73, 33.30, 52.41, 60.91, 102.55, 112.33, 119.02, 119.79, 120.10, 121.56, 112.47, 123.97, 124.46, 126.73, 128.11, 131.23, 131.83, 132.54, 132.89, 133.04, 136.92, 138.85, 148.85, 158.89, 168.66, 170.47.

*4.1.6.17. 2-{2-[(1H-Indole-2-carbonyl)-amino]-benzoylamino}-3-(4-nitro-phenyl)-propionic acid ethyl ester (**17f**).* Trituration with hot MeOH afforded the titled compound in 68% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.53; m.p. 206–207 °C; ^{1}H-NMR (DMSO-d_6) δ 1.16 (t, 3H, –CH_3); 3.34 (m, 2H, –C<u>H</u>$_2$–CH<); 4.15 (q, 2H, –CH_2–O–); 4.89 (m, 1H, –CH<); 6.94 (s, 1H, Ar); 7.09 (t, 1H, Ar); 7.23 (m, 2H, Ar); 7.48 (d, 1H, Ar); 7.57–7.80 (m, 5H, Ar); 8.14 (d, 2H, Ar); 8.63 (d, 1H, Ar); 9.37 (d, 1H, –N<u>H</u>–CH<); 11.93 (s, 1H, –NH–); 12.09 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 13.99, 35.78, 53.64, 60.99, 102.49, 112.50, 119.19, 119.95, 120.20, 121.73, 122.62, 123.25, 124.08, 126.90, 128.39, 130.52, 131.44, 132.73, 137.13, 139.14, 145.82, 146.31, 159.07, 168.94, 170.75.

*4.1.6.18. 2-{2-[(1H-Indole-2-carbonyl)-amino]-benzoylamino}-3-pyridin-3-yl-propionic acid ethyl ester (**18f**).* Trituration with cold diethyl ether afforded the titled compound in 68% yield. TLC (AcOEt/MeOH 2:1) - Rf: 0.85; m.p. 153–154 °C; ^{1}H-NMR (DMSO-d_6) δ 1.11 (t, 3H, –CH_3); 3.41 (m, 2H, –C<u>H</u>$_2$–CH<); 4.11 (q, 2H, –CH_2–O–); 4.91 (m, 1H, –CH<); 6.91 (s, 1H, Ar); 7.06–7.25 (m, 3H, Ar); 7.42–8.04 (m, 5H, Ar); 8.50–8.93 (m, 4H, Ar); 9.43 (d, 1H, –N<u>H</u>–CH<); 11.89 (s, 1H, –NH–); 12.05 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 14.81, 33.50, 53.84, 61.87, 103.25, 113.24, 119.85, 120.81, 120.97, 122.49, 123.44, 124.85, 127.01, 127.60, 129.19, 132.16, 133.55, 137.80, 138.02, 139.84, 141.49, 143.63, 146.42, 159.77, 169.65, 171.07.

*4.1.6.19. 3-Cyclohexyl-2-{2-[(1H-indole-2-carbonyl)-amino]-benzoylamino}-propionic acid ethyl ester (**19f**).* Trituration with hot MeOH afforded the titled compound in 47% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.79; m.p. 202 °C; ^{1}H-NMR (DMSO-d_6) δ 0.92–1.23 (m, 8H, cyclohexyl and –CH_3); 1.50–1.82 (m, 8H, cyclohexyl and –C<u>H</u>$_2$–C_6H_{11}); 4.14 (q, 2H, –CH_2–O–); 4.60 (m, 1H, >C<u>H</u>–CH_2–C_6H_{11}); 7.04 (s, 1H, Ar); 7.11 (d, 1H, Ar); 7.25 (m, 2H, Ar); 7.49 (d, 1H, Ar); 7.63 (t, 1H, Ar); 7.70 (d, 1H, Ar); 7.92 (d, 1H, Ar); 8.65 (d, 1H, Ar); 9.17 (d, 1H, –N<u>H</u>–CH<); 11.96 (s, 1H, –NH–); 12.24 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 13.91, 25.36, 25.54, 25.79, 31.33, 32.89, 33.61, 37.39, 50.35, 60.46, 102.37, 112.33, 119.49, 119.86, 120.03, 121.55, 122.47, 123.89, 126.77, 128.51, 131.36, 132.41, 136.95, 138.90, 158.97, 168.91, 172.06.

*4.1.7. General procedure for the synthesis of compounds **1–19***

A mixture of 4.0 mmol of the corresponding ethyl ester (compounds **1f-18f**) in water (25 mL) and tetrahydrofuran (25 mL) and in the presence of KOH (0.22 g, 4.0 mmol) was stirred at room temperature to 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.

*4.1.7.1. 2-{2-[(1H-Indole-2-carbonyl)-amino]-benzoylamino}-3-o-tolyl-propionic acid (**1**).* Trituration with hot MeOH afforded the titled compound in 87% yield. TLC (AcOEt/MeOH 2:1) - Rf: 0.46, m.p. 278 °C; ^{1}H-NMR (DMSO-d_6) δ 2.34 (s, 3H, –CH_3); 3.20 (m, 2H, –CH_2–); 4.75 (m, 1H, –CH<); 6.95 (s, 1H, Ar); 7.01–7.25 (m, 7H, Ar); 7.46 (d, 1H, Ar); 7.56 (t, 1H, Ar); 7.70 (d, 1H, Ar); 7.81 (d, 1H, Ar); 8.62 (d, 1H, Ar); 9.19 (d, 1H, –NH–CH<); 11.90 (s, 1H, –NH–); 12.21 (s, 1H, –NH–); 13.02 (b, 1H, –OH); ^{13}C-NMR (DMSO-d_6) δ 19.74, 34.49, 53.40, 103.37, 113.23, 119.94, 120.58, 120.93, 122.57, 123.23, 124.80, 126.29, 127.26, 127.68, 129.15, 130.27, 130.78, 132.25, 133.31, 136.77, 137.84, 139.96, 159.82, 169.49, 173.58. MS (ES) *m/z* 442 $[MH]^+$; MW 441.49 (calcd. for $C_{26}H_{23}N_3O_4$).

*4.1.7.2. 2-{2-[(1H-Indole-2-carbonyl)-amino]-benzoylamino}-3-p-tolyl-propionic acid (**2**).* Trituration with hot MeOH afforded the titled compound in 58% yield. TLC (AcOEt/MeOH 2:1) - Rf: 0.40; m.p. 273 °C; ^{1}H-NMR (DMSO-d_6) δ 2.13 (s, 3H, –CH_3); 3.21 (m, 2H, –CH_2–); 4.76 (m, 1H, –CH<); 6.96 (s, 1H, Ar); 7.07 (m, 3H, Ar); 7.24 (m, 4H, Ar); 7.49 (d, 1H, Ar); 7.60 (t, 1H, Ar); 7.75 (d, 1H, Ar); 7.84 (d, 1H, Ar); 8.66 (d, 1H, Ar); 9.17 (d, 1H, –NH–CH<); 11.94 (s, 1H, –NH–); 12.26 (s, 1H, –NH–); 13.02 (b, 1H, –OH); ^{13}C-NMR (DMSO-d_6) δ 20.35, 35.62, 54.06, 102.39, 112.31, 119.03, 119.63, 120.01, 121.67, 122.33, 123.89, 126.78, 128.22, 128.57, 128.74, 131.34, 132.41, 134.63, 135.20, 136.94, 139.07, 158.91, 168.51, 172.56. MS (ES) *m/z* 442 $[MH]^+$; MW 441.49 (calcd. for $C_{26}H_{23}N_3O_4$).

*4.1.7.3. 3-(4-Ethyl-phenyl)-2-{2-[(1H-indole-2-carbonyl)-amino]-benzoylamino}-propionic acid (**3**).* Trituration with hot 95% EtOH afforded the titled compound in 85% yield. TLC (AcOEt/MeOH 2:1) - Rf: 0.48; m.p. 265–266 °C; ^{1}H-NMR (DMSO-d_6) δ 1.01 (t, 3H, –CH_3); 2.40 (q, 2H, –CH_2–CH_3); 3.10 (m, 2H, –CH_2–CH<); 4.70 (m, 1H, –CH<); 6.92 (s, 1H, Ar); 7.06 (m, 3H, Ar); 7.19 (m, 4H, Ar); 7.41 (d, 1H, Ar); 7.56 (t, 1H, Ar); 7.70 (d, 1H, Ar); 7.80 (d, 1H, Ar); 8.60 (d, 1H, Ar); 9.10 (d, 1H, –NH–CH<); 11.85 (s, 1H, –NH–); 12.21 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 16.17, 28.42, 36.51, 54.98, 103.29, 113.21, 119.88, 120.52, 120.91, 122.57, 123.24, 124.79, 127.65, 128.26, 129.12, 129.65, 132.20, 133.33, 135.80, 137.80, 139.94, 142.41, 159.77, 169.41, 173.43; MS (ES) *m/z* 456 $[MH]^+$; MW 455.52 (calcd. for $C_{27}H_{25}N_3O_4$).

*4.1.7.4. 3-(4-tert-Butyl-phenyl)-2-{2-[(1H-indole-2-carbonyl)-amino]-benzoylamino}-propionic acid (**4**).* Trituration with hot MeOH afforded the titled compound in 62% yield. TLC (AcOEt/MeOH 2:1) - Rf: 0.56; m.p. 222 °C; ^{1}H-NMR (DMSO-d_6) 1.16 (s, 9H, –$C(CH_3)_3$); 3.17 (m, 2H, –CH_2–); 4.77 (m, 1H, –CH<); 7.02 (s, 1H, Ar); 7.10 (t, 1H, Ar); 7.18–7.27 (m, 6H, Ar); 7.49 (d, 1H, Ar); 7.60 (t, 1H, Ar); 7.75 (d, 1H, Ar); 7.86 (d, 1H, Ar); 8.66 (d, 1H, Ar); 9.18 (d, 1H, –NH–CH<); 11.94 (s, 1H, –NH–); 12.33 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 30.87, 33.83, 35.35, 53.99, 102.40, 112.31, 119.04, 119.65, 119.99, 121.67, 122.31, 123.88, 124.75, 126.79, 128.26, 128.44, 131.36, 132.41, 134.69, 136.95, 139.11, 148.43, 158.92, 168.64, 172.61. MS (ES) *m/z* 484 $[MH]^+$; MW 483.57 (calcd. for $C_{29}H_{29}N_3O_4$).

*4.1.7.5. 3-Biphenyl-4-yl-2-{2-[(1H-indole-2-carbonyl)-amino]-benzoylamino}-propionic acid (**5**).* Trituration with hot 95% EtOH afforded the titled compound in 50% yield. TLC (AcOEt/MeOH 2:1) - Rf: 0.48; m.p. 273–274 °C; ^{1}H-NMR (DMSO-d_6) δ 3.20 (m, 2H, –CH_2–); 4.80 (m, 1H, –CH<); 6.95 (s, 1H, Ar); 7.07 (t, 1H, Ar); 7.15–7.60 (m, 13H, Ar); 7.72 (d, 1H, Ar); 7.81 (d, 1H, Ar); 8.59 (d, 1H, Ar); 9.15 (d, 1H, –NH–CH<); 11.90 (s, 1H, –NH–); 12.25 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 36.49, 54.80, 103.29, 113.21, 119.96, 120.56, 120.91, 122.58, 123.27, 124.79, 127.11, 127.16, 127.66, 127.88, 129.12, 129.49, 130.33, 132.20, 133.34, 137.81, 137.96, 138.92, 139.93, 140.45, 159.77, 169.47, 173.38. MS (ES) *m/z* 504 $[MH]^+$; MW 503.56 (calcd. for $C_{31}H_{25}N_3O_4$).

*4.1.7.6. 2-{2-[(1H-Indole-2-carbonyl)-amino]-benzoylamino}-3-(2-methoxy-phenyl)-propionic acid (**6**).* Trituration with hot 95% EtOH afforded the titled compound in 59% yield. TLC (AcOEt/MeOH 2:1) - Rf: 0.60; m.p. 244 °C; ^{1}H-NMR (DMSO-d_6) δ 3.20 (m, 2H, –CH_2–); 3.84 (s, 3H, –CH_3); 4.84 (m, 1H, –CH<); 6.82 (t, 1H, Ar); 6.97 (m, 2H, Ar); 7.21–7.29 (m, 5H, Ar); 7.59 (m, 2H, Ar); 7.79 (m, 2H, Ar); 8.67 (d, 1H, Ar); 9.12 (d, 1H, –NH–CH<); 11.95 (s, 1H, –NH–); 12.31 (s, 1H, –NH–); 12.80 (b, 1H, –OH); ^{13}C-NMR (DMSO-d_6) δ 31.44, 52.34, 55.37, 102.64, 110.57, 112.53, 119.24, 119.82, 120.00, 120.23, 121.88, 122.50, 124.10, 125.56, 127.01, 128.05, 128.38, 130.76, 131.61, 132.58, 137.16, 139.31, 157.31, 159.14, 168.74, 172.96. MS (ES) *m/z* 458 $[MH]^+$; MW 457.49 (calcd. for $C_{26}H_{23}N_3O_5$).

*4.1.7.7. 2-{2-[(1H-Indole-2-carbonyl)-amino]-benzoylamino}-3-(3-methoxy-phenyl)-propionic acid (**7**).* Trituration with hot 95% EtOH afforded the titled compound in 65% yield. TLC (AcOEt/MeOH 2:1) - Rf: 0.48; m.p. 239 °C; ^{1}H-NMR (DMSO-d_6) δ 3.13 (m, 2H, –CH_2–); 3.61 (s, 3H, –CH_3); 4.75 (m, 1H, –CH<); 6.65 (d, 1H, Ar); 6.92 (m, 3H, Ar); 7.15 (m, 4H, Ar); 7.46 (d, 1H, Ar); 7.56 (t, 1H, Ar); 7.70 (d, 1H, Ar); 7.82 (d, 1H, Ar); 8.64 (d, 1H, Ar); 4–8.66 (m, 13H, Ar); 9.15 (d, 1H, –NH–CH<); 11.91 (s, 1H, –NH–); 12.28 (s, 1H, –NH–); 13.04 (b, 1H, –OH); ^{13}C-NMR (DMSO-d_6) δ 36.95, 54.88, 55.55, 103.36, 112.60, 113.25, 115.46, 119.88, 120.59, 120.95, 122.03, 122.60, 123.22, 124.83, 127.70, 129.17, 129.92, 132.28, 133.38, 137.86, 140.05, 140.21, 159.83, 169.44, 173.40. MS (ES) *m/z* 458 $[MH]^+$; MW 457.49 (calcd. for $C_{26}H_{23}N_3O_5$).

*4.1.7.8. 2-{2-[(1H-Indole-2-carbonyl)-amino]-benzoylamino}-3-(4-methoxy-phenyl)-propionic acid (**8**).* Trituration with hot 95% EtOH afforded the titled compound in 87% yield. TLC (AcOEt/MeOH 2:1) - Rf: 0.49; m.p. 259 °C; ^{1}H-NMR (DMSO-d_6) δ 3.15 (m, 2H, –CH_2–); 3.56 (s, 3H, –CH_3); 4.69 (m, 1H, –CH<); 6.78 (m, 2H, Ar); 6.96 (s, 1H, Ar); 7.06 (t, 1H, Ar); 7.22 (m, 4H, Ar); 7.46 (d, 1H, Ar); 7.56 (t, 1H, Ar); 7.71 (d, 1H, Ar); 7.82 (d, 1H, Ar); 8.63 (d, 1H, Ar); 9.12 (d, 1H, –NH–CH<); 11.90 (s, 1H, –NH–); 12.27 (s, 1H, –NH–); 12.95 (b, 1H, –OH); ^{13}C-NMR (DMSO-d_6) δ 36.11, 55.18, 55.52, 103.31, 113.21, 114.32, 119.92, 120.56, 120.91, 122.59, 123.24, 124.81, 127.69, 129.15, 130.43, 130.76, 132.26, 133.32, 137.84, 140.00, 158.53, 159.81, 169.45, 173.48. MS (ES) *m/z* 458 $[MH]^+$; MW 457.49 (calcd. for $C_{26}H_{23}N_3O_5$).

*4.1.7.9. 3-(4-Fluoro-phenyl)-2-{2-[(1H-indole-2-carbonyl)-amino]-benzoylamino}-propionic acid (**9**).* Trituration with hot MeOH afforded the titled compound in 82% yield. TLC (AcOEt/MeOH 2:1) - Rf: 0.54; m.p. 263–264 °C; ^{1}H-NMR (DMSO-d_6) δ 3.18 (m, 2H, –CH_2–); 4.74 (m, 1H, –CH<); 6.93 (s, 1H, Ar); 7.04–7.47 (m, 8H, Ar); 7.55 (t, 1H, Ar); 7.70 (d, 1H, Ar); 7.79 (d, 1H, Ar); 8.62 (d, 1H, Ar); 9.14 (d, 1H, –NH–CH<); 11.89 (s, 1H, –NH–); 12.23 (s, 1H, –NH–); 13.00 (b, 1H, –OH); ^{13}C-NMR (DMSO-d_6) δ 36.08, 54.88, 103.27, 113.21, 115.41, 115.83, 119.81, 120.55, 120.90, 122.58, 123.24, 124.79, 127.67, 129.10, 131.55, 131.70, 132.23, 133.36, 134.74, 134.80, 137.83, 140.00, 159.25, 159.79, 164.06, 169.44, 173.28; MS (ES) *m/z* 446 $[MH]^+$; MW 445.45 (calcd. for $C_{25}H_{20}FN_3O_4$).

*4.1.7.10. 3-(2-Chloro-phenyl)-2-{2-[(1H-indole-2-carbonyl)-amino]-benzoylamino}-propionic acid (**10**).* Trituration with hot 95% EtOH afforded the titled compound in 71% yield. TLC (AcOEt/MeOH 2:1) - Rf: 0.58; m.p. 281 °C; ^{1}H-NMR (DMSO-d_6) δ 3.30 (m, 2H, –CH_2–); 4.86 (m, 1H, –CH<); 6.92 (s, 1H, Ar); 7.06–7.21 (m, 5H, Ar); 7.36–7.47 (m, 3H, Ar); 7.55 (t, 1H, Ar); 7.71 (d, 1H, Ar); 7.79 (d, 1H, Ar); 8.61 (d, 1H, Ar); 9.22 (d, 1H, –NH–CH<); 11.90 (s, 1H, –NH–); 12.16 (s, 1H, –NH–); 13.10 (b, 1H, –OH); ^{13}C-NMR (DMSO-d_6) δ 34.96, 52.71, 103.38, 113.21, 119.90, 120.59, 120.93, 122.58, 123.24, 124.81, 127.67, 129.06, 129.22, 129.92, 132.21, 132.43, 133.34, 134.00, 136.03, 137.83, 139.94, 159.79, 169.45, 173.12. MS (ES) *m/z* 462, 464 $[MH]^+$; MW 461.91 (calcd. for $C_{25}H_{20}ClN_3O_4$).

*4.1.7.11. 3-(3-Chloro-phenyl)-2-{2-[(1H-indole-2-carbonyl)-amino]-benzoylamino}-propionic acid (**11**).* Trituration with hot 95% EtOH afforded the titled compound in 73% yield. TLC (AcOEt/MeOH 2:1) - Rf: 0.52; m.p. 248 °C; ^{1}H-NMR (DMSO-d_6) δ 3.18 (m, 2H, –CH_2–); 4.78 (m, 1H, –CH<); 6.93 (s, 1H, Ar); 7.03–7.26 (m, 6H, Ar); 7.47 (m, 2H, Ar); 7.56 (t, 1H, Ar); 7.69 (d, 1H, Ar); 7.78 (d, 1H, Ar); 8.63 (d, 1H, Ar); 9.17 (d, 1H, –NH–CH<); 11.90 (s, 1H, –NH–); 12.19 (s, 1H, –NH–); 13.05 (b, 1H, –OH); ^{13}C-NMR (DMSO-d_6) δ 36.52, 54.52, 103.33, 113.22, 119.87, 120.58, 120.91, 122.58, 123.23, 124.79, 127.14, 127.68, 128.52, 129.06, 129.74, 130.63, 132.25, 133.37, 133.47, 137.84, 139.99, 141.20, 159.81, 169.41, 173.12. MS (ES) *m/z* 462, 464 $[MH]^+$; MW 461.91 (calcd. for $C_{25}H_{20}ClN_3O_4$).

*4.1.7.12. 3-(4-Chloro-phenyl)-2-{2-[(1H-indole-2-carbonyl)-amino]-benzoylamino}-propionic acid (**12**).* Trituration with hot 95% EtOH afforded the titled compound in 75% yield. TLC (AcOEt/MeOH 2:1) - Rf: 0.51; m.p. 282 °C; ^{1}H-NMR (DMSO-d_6) δ 3.14 (m, 2H, –CH_2–); 4.77 (m, 1H, –CH<); 6.96 (s, 1H, Ar); 7.03–7.38 (m, 7H, Ar); 7.46 (d, 1H, Ar); 7.56 (t, 1H, Ar); 7.71 (d, 1H, Ar); 7.80 (d, 1H, Ar); 8.64 (d, 1H, Ar); 9.17 (d, 1H, –NH–CH<); 11.90 (s, 1H, –NH–); 12.26 (s, 1H, –NH–); 13.05 (b, 1H, –OH); ^{13}C-NMR (DMSO-d_6) δ 36.24, 54.70, 103.31, 113.23, 119.78, 120.59, 120.91, 122.59, 123.24, 124.79, 127.70, 128.84, 129.10, 131.67, 131.91, 132.26, 133.38, 137.66, 137.85, 140.04, 159.83, 169.47, 173.22. MS (ES) *m/z* 462, 464 $[MH]^+$; MW 461.91 (calcd. for $C_{25}H_{20}ClN_3O_4$).

*4.1.7.13. 3-(3,5-Dichloro-phenyl)-2-{2-[(1H-indole-2-carbonyl)-amino]-benzoylamino}-propionic acid (**13**).* Trituration with hot 95% EtOH afforded the titled compound in 67% yield. TLC (AcOEt/MeOH 2:1) - Rf: 0.46; m.p. 277 °C; ^{1}H-NMR (DMSO-d_6) δ 3.20 (m, 2H, –CH_2–); 4.78 (m, 1H, –CH<); 6.87 (s, 1H, Ar); 7.06 (t, 1H, Ar); 7.18 (m, 3H, Ar); 7.38 (s, 2H, Ar); 7.44 (d, 1H, Ar); 7.56 (t, 1H, Ar); 7.68 (d, 1H, Ar); 7.75 (d, 1H, Ar); 8.61 (d, 1H, Ar); 9.13 (d, 1H, –NH–CH<); 11.88 (s, 1H, –NH–); 12.05 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 36.30, 54.02, 103.32, 113.21, 119.95, 120.56, 120.92, 122.61, 123.30, 124.80, 126.84, 127.62, 128.75, 132.18, 133.42, 134.29, 137.79, 139.84, 142.96, 159.76, 169.33, 172.82. MS (ES) *m/z* 496, 498 $[MH]^+$; MW 496.35 (calcd. for $C_{25}H_{20}Cl_2N_3O_4$).

*4.1.7.14. 3-(2,6-Dichloro-phenyl)-2-{2-[(1H-indole-2-carbonyl)-amino]-benzoylamino}-propionic acid (**14**).* Trituration with hot 95% EtOH afforded the titled compound in 83% yield. TLC (AcOEt/MeOH 2:1) - Rf: 0.53; m.p. 287 °C; ^{1}H-NMR (DMSO-d_6) δ 3.53 (m, 2H, –CH_2–); 4.74 (m, 1H, –CH<); 6.90 (s, 1H, Ar); 7.04–7.26 (m, 4H, Ar); 7.37–7.47 (m, 3H, Ar); 7.57 (t, 1H, Ar); 7.71 (d, 1H, Ar); 7.81 (d, 1H, Ar); 8.61 (d, 1H, Ar); 9.28 (d, 1H, –NH–CH<); 11.89 (s, 1H, –NH–); 12.14 (s, 1H, –NH–); 13.00 (b, 1H, –OH); ^{13}C-NMR (DMSO-d_6) δ 32.77, 52.31, 103.36, 113.23, 120.09, 120.65, 120.96, 122.55, 123.30, 124.83, 127.63, 129.10, 129.96, 132.18, 133.28, 134.54, 135.98, 137.81, 139.86, 159.80, 169.38, 172.44. MS (ES) *m/z* 496, 498 $[MH]^+$; MW 496.35 (calcd. for $C_{25}H_{20}Cl_2N_3O_4$).

*4.1.7.15. 4-(2-Carboxy-2-{2-[(1H-indole-2-carbonyl)-amino]-benzoylamino}-ethyl)-benzoic acid (**15**).* Trituration with cold CH_2Cl_2 afforded the titled compound in 45% yield. TLC (AcOEt/MeOH 2:1) - Rf: 0.25; m.p. 280–281 °C; ^{1}H-NMR (DMSO-d_6) δ 3.23 (m, 2H, –CH_2–); 4.76 (m, 1H, –CH<); 6.94 (s, 1H, Ar); 7.05 (t, 1H, Ar); 7.19 (m, 2H, Ar);

7.43–7.85 (m, 8H, Ar); 8.61 (d, 1H, Ar); 9.17 (d, 1H, –NH–CH<); 11.87 (s, 1H, –NH–); 12.26 (s, 1H, –NH–); 12.89 (b, –OH); ^{13}C-NMR (DMSO-d_6) δ 35.88, 53.64, 102.34, 112.31, 118.76, 119.63, 119.98, 121.69, 122.34, 123.87, 126.76, 128.20, 128.83, 129.08, 130.40, 131.34, 132.50, 136.91, 139.15, 143.07, 158.90, 166.89, 168.55, 172.21. MS (ES) *m/z* 472 $[MH]^+$; MW 471.47 (calcd. for $C_{26}H_{21}N_3O_6$).

*4.1.7.16. 2-{2-[(1H-Indole-2-carbonyl)-amino]-benzoylamino}-3-(2-nitro-phenyl)-propionic acid (**16**).* Trituration with cold CH_2Cl_2 afforded the titled compound in 95% yield. TLC (AcOEt/MeOH 2:1) - Rf: 0.41; m.p. 253 °C; ^{1}H-NMR (DMSO-d_6) δ 3.40 (m, 2H, –CH_2–); 4.94 (m, 1H, –CH<); 6.87 (s, 1H, Ar); 7.03–7.35 (m, 4H, Ar); 7.42–7.58 (m, 4H, Ar); 7.72 (m, 2H, Ar); 7.87 (d, 1H, Ar); 8.58 (d, 1H, Ar); 9.18 (d, 1H, –NH–CH<); 11.85 (s, 1H, –NH–); 12.02 (s, 1H, –NH–); 13.10 (b, 1H, –OH); ^{13}C-NMR (DMSO-d_6) δ 34.46, 52.90, 103.50, 113.21, 119.92, 120.62, 121.03, 122.50, 123.40, 124.90, 125.29, 127.61, 128.88, 132.08, 133.06, 133.37, 133.72, 133.83, 137.76, 139.70, 149.69, 159.75, 169.33, 172.80. MS (ES) *m/z* 473 $[MH]^+$; MW 472.46 (calcd. for $C_{25}H_{20}N_4O_6$).

*4.1.7.17. 2-{2-[(1H-Indole-2-carbonyl)-amino]-benzoylamino}-3-(4-nitro-phenyl)-propionic acid (**17**).* Trituration with hot 95% EtOH afforded the titled compound in 84% yield. TLC (AcOEt/MeOH 2:1) - Rf: 0.49; m.p. 243 °C; ^{1}H-NMR (DMSO-d_6) δ 3.32 (m, 2H, –CH_2–); 4.86 (m, 1H, –CH<); 6.89 (s, 1H, Ar); 7.05 (t, 1H, Ar); 7.19 (m, 2H, Ar); 7.44 (d, 1H, Ar); 7.51–7.71 (m, 4H, Ar); 7.80 (d, 1H, Ar); 8.09 (d, 2H, Ar); 8.60 (d, 1H, Ar); 9.25 (d, 1H, –NH–CH<); 11.87 (s, 1H, –NH–); 12.16 (s, 1H, –NH–); 13.10 (b, 1H, –OH); ^{13}C-NMR (DMSO-d_6) δ 35.81, 53.38, 102.33, 112.34, 118.89, 119.70, 120.01, 121.67, 122.40, 123.07, 123.90, 126.76, 128.25, 130.31, 131.31, 132.53, 136.96, 139.11, 146.05, 146.18, 158.88, 168.59, 172.01. MS (ES) *m/z* 473 $[MH]^+$; MW 472.46 (calcd. for $C_{25}H_{20}N_4O_6$).

*4.1.7.18. 2-{2-[(1H-Indole-2-carbonyl)-amino]-benzoylamino}-3-pyridin-3-yl-propionic acid (**18**).* Trituration with hot 95% EtOH afforded the titled compound in 51% yield. TLC (AcOEt/MeOH 2:1) - Rf: 0.19; m.p. 271 °C; ^{1}H-NMR (DMSO-d_6) δ 3.12 (m, 2H, –CH_2–); 4.78 (m, 1H, –CH<); 6.92 (s, 1H, Ar); 7.07 (t, 1H, Ar); 7.23 (m, 3H, Ar); 7.45 (d, 1H, Ar); 7.57 (t, 1H, Ar); 7.74 (m, 3H, Ar); 8.30 (d, 1H, Ar); 8.51 (s, 1H, Ar); 8.61 (d, 1H, Ar); 9.17 (d, 1H, –NH–CH<); 11.89 (s, 1H, –NH–); 12.18 (s, 1H, –NH–); 13.10 (b, 1H, –OH); ^{13}C-NMR (DMSO-d_6) δ 34.17, 54.35, 103.34, 113.21, 119.89, 120.62, 120.91, 122.55, 123.27, 123.95, 124.79, 127.66, 129.02, 132.23, 133.35, 134.16, 137.24, 137.83, 139.94, 148.38, 150.93, 159.79, 169.39, 173.00. MS (ES) *m/z* 429 $[MH]^+$; MW 428.45 (calcd. for $C_{24}H_{20}N_4O_4$).

*4.1.7.19. 3-Cyclohexyl-2-{2-[(1H-indole-2-carbonyl)-amino]-benzoylamino}-propionic acid (**19**).* Trituration with hot MeOH afforded the titled compound in 55% yield. TLC (AcOEt/MeOH 2:1) - Rf: 0.58; m.p. 222 °C; ^{1}H-NMR (DMSO-d_6) δ 0.94–1.14 (m, 5H, cyclohexyl); 1.65–1.82 (m, 8H, cyclohexyl and –CH_2–C_6H_{11}); 4.61 (m, 1H, >CH–CH_2–C_6H_{11}); 7.08 (s, 1H, Ar); 7.13 (d, 1H, Ar); 7.26 (m, 2H, Ar); 7.51 (d, 1H, Ar); 7.63 (t, 1H, Ar); 7.75 (d, 1H, Ar); 7.96 (d, 1H, Ar); 8.69 (d, 1H, Ar); 9.07 (d, 1H, –NH–CH<); 11.96 (s, 1H, –NH–); 12.41 (s, 1H, –NH–); 12.80 (b, 1H, –OH); ^{13}C-NMR (DMSO-d_6) δ 25.38, 25.57, 25.83, 31.25, 33.04, 33.70, 37.55, 50.09, 102.37, 112.32, 119.39, 119.80, 120.01, 121.64, 122.40, 123.88, 126.80, 128.49, 131.42, 132.35, 136.96, 139.05, 159.00, 168.74, 173.65. MS (ES) *m/z* 434 $[MH]^+$; MW 433.51 (calcd. for $C_{25}H_{27}N_3O_4$).

*4.1.8. 1H-Indole-2-carboxylic acid [2-(2-oxo-1,2,3,4-tetrahydro-quinolin-3-ylcarbamoyl)-phenyl]-amide (**20**)*

To a solution of 0.500 g (1.06 mmol) of compound **16** in 150 ml of THF, 30 mg of 10% Pd/C is added and the reaction mixture is stirred until completion (TLC control). The reaction mixture is filtered through celite and evaporated, obtaining the titled compound in 85% yield. TLC (AcOEt/n-hexane 1:1) - Rf: 0.23; m.p. >300 °C; ^{1}H-NMR (DMSO-d_6) δ 3.10 (m, 2H, –CH_2–); 4.85 (m, 1H, –CH<); 6.91–7.26 (m, 8H, Ar); 7.44 (d, 1H, Ar); 7.64 (m, 2H, Ar); 7.95 (d, 1H, Ar); 8.66 (d, 1H, Ar); 9.11 (d, 1H, –NH–CH<); 10.43 (s, 1H, –NH–); 11.89 (s, 1H, –NH–); 12.51 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 31.63, 49.01, 103.43, 113.21, 115.86, 120.07, 120.73, 120.89, 122.56, 123.05, 123.38, 123.65, 124.79, 127.68, 128.34, 128.85, 129.08, 132.30, 133.41, 137.79, 138.17, 140.13, 159.88, 169.00, 169.32. MS (ES) *m/z* 425 $[MH]^+$; MW 424.46 (calcd. for $C_{25}H_{20}N_4O_3$).

4.2. 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(sulfated), [^{125}I]Bolton and Hunter labelled-specific activity 2000 Ci/mol) was purchased from Amersham Pharmacia Biothech (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 [35].

4.2.1. [^{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. [18]. 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.

4.2.2. [^{125}I]BH-CCK-8 receptor binding assay in guinea pig cerebral cortices

Membranes from guinea pig cerebral cortices, were prepared as previously described [18]. Protein concentration was determined according to Bradford [36], 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 per 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. Non-specific 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).

4.3. Statistical analysis

In QSAR equation n is the number of data points, *r* is the correlation coefficient, s is the standard deviation, Q is a measure of the quality of fit calculated as described by Cramer et al. [37] and the data within the parenthesis are for the 95% confidence intervals. The molar refractivities were determined using the program C-QSAR [19].

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