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Original article

Anthranilic acid based CCK_1 antagonists: the 2-indole moiety may represent a "needle" according to the recent homonymous concept

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Abstract

Recently we described an innovative class of non-peptide CCK_1 antagonists keeping appropriate pharmacophoric groups on the anthranilic acid employed as a molecular scaffold. The lead compound obtained, **VL-0395**, characterized by the presence of Phe and the 2-indole moiety at the C- and N-termini of anthranilic acid, respectively, is endowed with submicromolar affinity towards CCK_1 receptors. Thus, we have prepared and tested on CCK receptors a library of **VL-0395** analogues in order to investigate the precise topological and essential key interactions of the 2-indole group of the lead with the CCK_1 receptor. The obtained results confirm that this group establishes very specific interactions with this receptor sub-site and may be viewed as a "needle" group.

Keywords: Cholecystokinin; CCK_1; Antagonist; Anthranilic acid

1. Introduction

In the last decade cholecystokinin (CCK) has become an attractive therapeutic target and has been the subject of intense research aimed at discovering potent and selective CCK-ligands [1,2]. We believe that different structural classes of CCK_1 receptor antagonists can be viewed as the result of a design strategy that combines the concepts of both "privileged structure" and the "needle" approach. The term "privileged structure" was introduced by Evans et al. [3] describing the benzodiazepine-based CCK_1 antagonists [4]. In their definition a privileged structure, such as the benzodiazepine nucleus, "is a single molecular framework able to provide ligands for diverse receptors". In fact, benzodiazepines are found in several types of CNS agents and this substructure is present in ligands of both ion channel and G-protein coupled receptors (GPCRs).

In contrast to the "privileged structure" concept, the term "needle" [5] was used in the literature to indicate a part of an active molecule showing very specific interactions towards one particular biological target.

It is interesting to note that the most potent benzodiazepine-based CCK_1 antagonist (devazepide) is characterized by the presence of the 2-indole moiety [6].

Within the CCK area the same moiety has been described for several CCK_1 antagonists such as tricyclic benzodiazepines [7,8], benzolactams [9], β-carbolines [10], aminoacid [11] and amino-thiazole derivatives [12] as well as for the hybrid antagonists [13,14], suggesting its possible role as a "needle".

Recently, we described an innovative class of non-peptide CCK_1 antagonists keeping appropriate pharmacophoric groups on the anthranilic acid unit employed as a molecular scaffold. The lead compound obtained, **VL-0395** (Fig. 1), is characterized by the presence of Phe and 2-indole moiety at the C- and N-termini of anthranilic acid, respectively, and is endowed with submicromolar affinity towards CCK_1 receptors [15].

In that preliminary study the indole-2-carbonyl group imparts the higher affinity (IC_{50} = 197 nM), whereas other indole isomers and aromatic, bi-aromatic or aliphatic substituents resulted in a sharp decrease in the affinity. This fact suggests that this receptor binding sub-site imposes a high degree of conformational restrictions.

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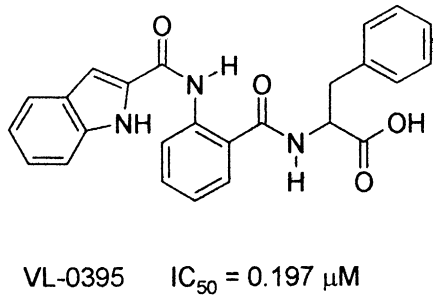


Fig. 1. Structure of the anthranilic acid derivative **VL-0395**.

Hence, we have hypothesized that the 2-indole moiety is able to establish very specific interactions with the receptor and may represent effectively a "needle" type group even in this new class of CCK_1 receptor antagonists.

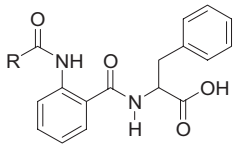
In order to verify our hypothesis, we decided to investigate on the precise topological and essential key interactions of the 2-indole group of **VL-0395** with the CCK_1 receptor. To this end we prepared a library of **VL-0395** analogues reported in Table 1.

2. Chemistry

The general synthesis of anthranilic acid derivatives **1–23** is shown in Fig. 2.

Ortho-amino benzoylation of the DL-phenylalanine ethyl ester with isatoic anhydride gave the intermediate **24** in 80% yield, which in turn was condensed, employing different coupling protocols, with the corresponding acids to give the esters **1a–23a**. Compounds **1a, 4a, 6a, 8a** and **10a–21a** were obtained via acyl chloride formation by a standard method using PCl_5 in dry dichloromethane except for compounds **9a** and **17a** for which the commercially available acyl chlorides were used. Compounds **22a** and **23a** were obtained from the corresponding cycloalka[b]pyrrole-2-carboxylic acids, which are highly sensitive to acidic mediums, by in situ

Table 1
Structure of target compounds and CCK receptors binding data



Compound	R	IC_{50} [a] (μM)	
		Rat pancreatic acini (CCK_1) [b]	Guinea pig brain cortex (CCK_2) [b]
VL-0395	2-Indolyl	0.197 (0.131–0.298)	16.40
1	2-(1-Methyl)-indolyl	0.350 (0.218–0.563)	10.00
2	2-Benzimidazolyl	3.39 (1.68–6.86)	10.31
3	2-Benzofuranyl	0.891 (0.432–1.839)	13.70
4	2-Benzo[b]thiophenyl	1.12 (0.93–1.34)	10.80
5	2-Indenyl	2.53 (0.98–6.47)	IN [c]
6	2-(3-Methyl)-indenyl	3.78 (1.63–8.77)	13.70
7	1-Acetylamino-2-phenylethenyl	12.72 (7.54–21.47)	19.30
8	(*E*)-2-(*o*-Nitro)-phenylethenyl	IN [d]	46% ISB [e]
9	(*E*)-2-Phenylethenyl	4.01	49% ISB [e]
10	2-Quinolinyl	48% ISB [c]	14.6
11	3-Quinolinyl	3.13 (2.16–4.54)	18.7
12	4-Quinolinyl	IN [d]	15.00
13	1-Isoquinolinyl	13.29 (5.83–30.28)	53% ISB [e]
14	2-(1-Methyl)-pyrrolyl	21% ISB [d]	NT
15	2-Pyrrolyl	IN [d]	NT
16	2-Furanyl	24% ISB [d]	27% ISB [e]
17	2-Thiophenyl	2.56	24% ISB [e]
18	2-Pyridinyl	IN [d]	31% ISB [e]
19	3-Pyridinyl	IN [d]	44% ISB [e]
20	4-Pyridinyl	IN [d]	26% ISB [e]
21	2-Pyrazinyl	IN [d]	IN [e]
22	Cyclohexa[b]pyrrole-2-yl	1.20	70.00
23	Cyclopenta[b]pyrrole-2-yl	13.06	60.50

[a] IC_{50}, μM concentration and $P = 0.05$ fiducial limits required to inhibit by 50% the specific binding of 25 pM [^{125}I]-(BH)-CCK8; % ISB, percentage inhibition of specific binding at the maximal concentration tested.

[b] Values without fiducial limits were obtained from not more than two experiments; IN, inactive; NT, not tested.

[c] 10 μM.

[d] 3 μM.

[e] 30 μM.

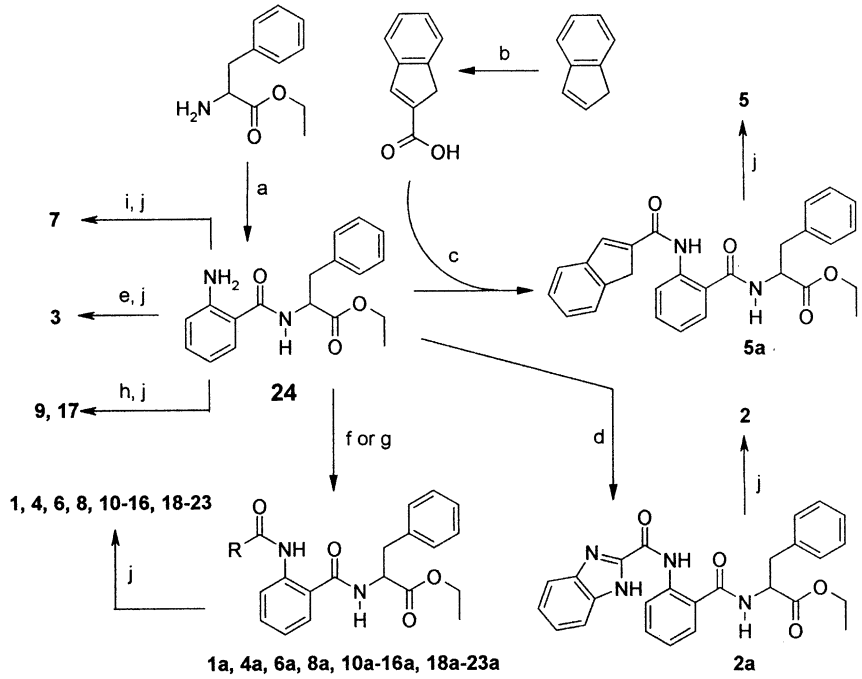


Fig. 2. Synthesis of compounds **1–23**. Reagents and conditions: (a) isatoic anhydride, Et_3N, AcOEt; (b) oxalyl dibromide; (c) 2-chloro-1-methylpyridinium iodide (Mukaiyama reagent), Et_3N, dry CH_2Cl_2; (d) 2-trichloromethyl-1*H*-benzimidazole, Et_3N, EtOH; (e) benzofuran-2-carboxylic acid, 1,1′-carbonyldiimidazole, dry THF; (f) RCOOH, PCl_5, dry CH_2Cl_2, pyridine; (g) RCOOH, $SOCl_2$, DMF, pyridine; (h) RCOCl, pyridine; (i) RCOOH, iBuOCOCl, Et_3N, dry CH_2Cl_2; (j) NaOH, THF/H_2O 1:1.

activation using $SOCl_2$ in pyridine in the presence of a catalytic amount of DMF [16]. The above pyrrole carboxylic acids were prepared by $LiOH{\cdot}H_2O$ catalyzed hydrolysis of the corresponding ethyl esters [17]. Derivative **7a** was obtained by coupling the intermediate **24** with the corresponding acid, which was previously activated by the mixed anhydride method. Indene-2-carboxylic acid was synthesized from indene and oxalyl dibromide following a known procedure [18]. The subsequent condensation with the intermediate **24** to afford ester **5a** was achieved using the Mukaiyama reagent [19]. Benzofuran derivative **3a** was obtained from **24** and the corresponding acid using carbonyldiimidazole (CDI) as a coupling agent. Since benzimidazole-2-carboxylic acid was not commercially available, our choice fell upon its synthetic equivalent 2-trichloromethylbenzimidazole. The reactivity of the latter is well established and it can be used as a precursor of benzimidazole-2-carboxylic acid itself [20] or used directly for condensation reactions with amines, affording amides [21]. For the reaction involving **24** and giving **2a**, we followed the procedure described by Garuti et al. [22]. The free acids **1–23** were obtained in nearly quantitative yields by base catalyzed hydrolysis of the corresponding ethyl esters **1a–23a**.

3. Biological evaluation

Compounds **1–23** were evaluated for their ability to displace [^{125}I] (BH)-CCK8 from isolated rat pancreatic acini (CCK_1) and guinea pig cerebral cortex membranes (CCK_2) according to established protocols [23]. Binding affinities expressed as IC_{50} or as percentage of inhibition (ISB%) determined at the highest used dose (3, 10, or 30 μM, as indicated) are reported in Table 1 along with those obtained for the lead compound **VL-0395**.

4. Results

As previously observed [15], all the tested compounds showed low affinity towards the central CCK_2 receptor subtype confirming their preference to the CCK_1 receptors.

In particular compound **1**, obtained by masking the indolic NH group of **VL-0395** with a methyl group, possesses an affinity similar to that of the lead compound, suggesting that the contribution of a hydrogen bond formation is relatively modest.

However, the importance of the presence of a nitrogen atom is illustrated by the decrease in affinity observed for indene derivative **5**; in this case its lower affinity may also be due to the absence of the π-*excessive* system.

The IC_{50} ratio between compounds **6** and **5** is roughly similar to that observed for the corresponding methylated compound **1** and the non-methylated reference compound. These results indicate the same effect due to the presence of the methyl group.

For further exploration on the role of the specific hydrogen bond in the receptor binding, the benzimidazole ring was chosen as the aza-bioisoster (compound **2**) of the indole moiety. In this case, the investigated NH group can be either

in *cis* or *trans*-like position with respect to the anthranilic nitrogen by tautomerism. Moreover, as a consequence of the presence of this second hydrogen-free nitrogen atom, the N–H bond of benzimidazole compared to the indolic NH has a more pronounced polarization, higher acidity [20,24] and is thought to be a better proton donor group.

The proposed replacement of the 2-indole ring with 2-benzimidazole has already been reported with successes in different fields of medicinal chemistry such as the NMDA receptors antagonists [25] and renin-angiotensin system inhibitors [26]. In this case, the introduction of the benzimidazole (compound **2**) instead of indole in **VL-0395** was less successful showing an IC_{50} at least 15-fold greater.

Then, the effect of the replacement of the indolic NH group with heteroatoms characterized by different hydrogen bond acceptor ability was investigated employing the benzofuran and benzothiophene heterocycles (**3** and **4**). The fact that the binding affinity of these two compounds was lower than that of **VL-0395** suggests that the receptor appears to prefer (moderately) a functional group, which serves as a hydrogen bond donor.

Compounds **7–9** are characterized by the presence of the cinnamoyl moiety, which can be envisioned as structurally similar to the indolyl group of the lead. Although the structural similarity of the *E*-cinnamoyl moiety to the 2-indole group, compound **9** showed a reduced level of CCK_1 affinity by a factor of 20 with respect to that of **VL-0395**. It is interesting to note that the substitution of the 2-indole moiety of the potent pyrrolo-1,4-benzodiazepine type CCK_1 antagonist FK-480 [8] with the *E*-cinnamoyl moiety gave rise to an equipotent compound.

With compounds **10–13** the π-*excessive* pyrrole ring of the 2-indole moiety of the lead was replaced by the π-*deficient* six termini ring of pyridine. Here, although no significant affinity was observed for these derivatives, the 3-quinoline system displayed the better affinity within this subset of compounds.

Finally, compounds **14–21** were prepared to investigate the binding contribute of the fused benzene ring of some of the above tested hetero-bicyclic systems. The sharp decrease of binding affinity for the selected compounds **14–20** containing the same pentatomic or hexatomic heterocyclic moieties of some bicyclic systems reported above, indicated that the fused benzene ring is crucial for the optimum steric and/or lipophilic requirement for this binding site. An additional proof of the importance of the fused benzene ring of the indole moiety of **VL-0395** was given with compounds **22** and **23**. In fact, the substitution of the benzene ring with saturated rings such as cyclohexyl and cyclopentyl was associated with a decrease in affinity.

5. Discussion and molecular modeling

The first criterion for the selection of the substituents was to fulfil the conformational preferences of the lead compound. In fact, from Fig. 3 which reports an overlay of the higher affinity ligands **1–6** and **VL-0395**, carried out by superimposing the common phenylalanine residues, it is clear that the overall shape of the molecules closely resembles the geometry of the lead compound, which has been previously described [15].

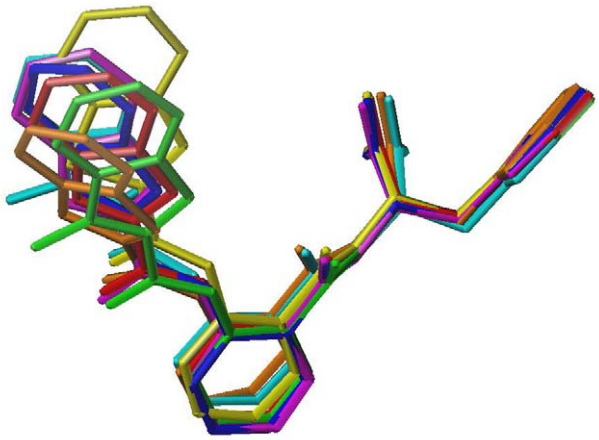


Fig. 3. Overlay of the higher affinity compounds **1–6** and **VL-0395** (heavy atoms only for clarity). **VL-0395**, red; Compound **1**, green; Compound **2**, blue; Compound **3**, magenta; Compound **4**, yellow; Compound **5**, orange and Compound **6**, cyan.

The intra-molecular hydrogen bond at the anthranilic core of the molecules is conserved, and plays the key role in determining the U-shaped conformation of the ligands. In all the molecules the phenylalanine aromatic ring lies on the same side of the N-terminal substituent, and the distance between the two aromatic ends of the molecules is very similar.

The same conformational preference of **VL-0395**, in which the indole and anthranilic nitrogens are in a *trans*-like position, is also observed in all the nitrogen heterocyclic compounds. On the contrary, the oxygen of the furane derivative (compound **3**) adopts a *cis*-like position with respect to the anthranilic nitrogen, and is thus placed on the internal side of the molecule. The sulfur atom of the thiophene ligand (compound **4**) does not show any conformational preference, and the two *trans*- and *cis*-like conformations are almost at the same energy.

As to the charge distribution, a major difference is observed between the sulfur and the other heterocyclic systems: in the nitrogen and oxygen compounds a negative charge is placed on the heteroatom, while the sulfur atom of the thiophene derivative bears a rather high positive charge (≈ +0.6).

The benzimidazole ligand has been optimized in both the neutral and the N-protonated cationic form, since the p*K* value [27] of the benzimidazole system allows for the possibility of a significant fraction of protonated molecules in the receptor microenvironment, as well as the presence of a large fraction of zwitterionic form (cationic at the heterocyclic system) at pH values very close to neutrality. The geometry of the cation is very different from the conformation of the

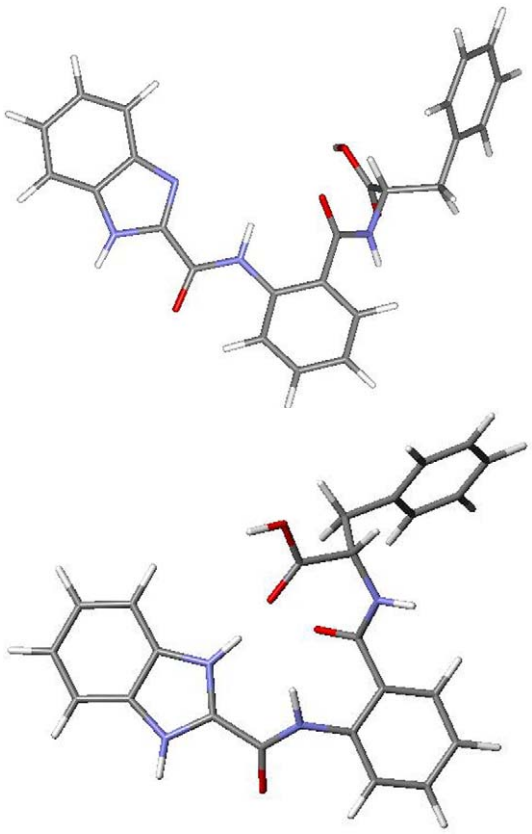


Fig. 4. Lowest energy conformations of benzimidazole derivative **2** in the neutral (top) and protonated (bottom) form.

neutral molecule, and this appears to be due to a strong interaction between the carboxylic end of the molecule and the charged ring (Fig. 4).

An intra-molecular hydrogen bond between the extra proton of the imidazole ring and the carboxylic oxygen is allowed in this conformation. This feature, together with the difference in charge distribution and electrostatic potential of the cation could explain the decrease of affinity for this compound.

6. Conclusions

In this paper we described a focused compound library targeted to evaluate the possible "needle" role for the 2-indole moiety of the new anthranilic acid based CCK_1 antagonists. From this study, it is evident that every single modification of the 2-indole moiety caused, to a greater or lesser extent, a decrease in binding affinity of the initial lead.

These findings suggest that keeping the correct orientation of the substituent in order to fulfil the conformational restrains of this receptor pocket, the major affinity of the 2-indole ring results from a balanced combination of aromatic (π-*excessive* system), hydrophobic and hydrogen bond interactions and could not be mimicked by similar heterocyclic systems.

Although this group has already been studied in different fields of medicinal chemistry, we believe that its "needle" role is better stressed in the CCK_1 receptor area. Moreover, it is interesting to note that the "needle" role of the 2-indole is much more evident on this series in comparison to the 1,4-benzodiazepine derivatives. In fact, the difference in binding affinity between the **VL-0395** and the proposed compounds was more pronounced than that observed for the 1,4-benzodiazepines bearing the same substituents [3,8].

In conclusion, considering that the identification either of an appropriate lead compound and its essential structural elements for a specific biological effect are vital steps for any pharmaceutical research project, this study clearly shows that the 2-indole moiety establishes very specific interactions with this receptor sub-site and may be viewed as "needle" group.

7. Experimental

7.1. 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 using $CDCl_3$ or $(CD_3)_2SO$ as solvent. Chemical shifts were reported as parts per million (ppm, δ units) downfield from an internal Me_4Si standard. Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet and b, broad. Spectral data are consistent with assigned structures. Mass spectra were recorded on an API-1 Perkin–Elmer SCIEX spectrometer by electrospray ionisation (ES).

7.1.1. 2(R,S)-(2-Amino-benzoylamino)-3-phenyl-propionic acid ethyl ester (*24*)

A suspension of DL-phenylalanine ethyl ester hydrochloride (5.00 g, 21.8 mmol) in 150 ml of ethyl acetate was treated with triethylamine (3.07 ml, 21.8 mmol) followed by isatoic anhydride (3.55 g, 21.8 mmol). The resulting mixture was refluxed under stirring for 2 h, cooled to room temperature and filtered. The organic phase was washed with 1 M NaOH (2 × 50 ml), water (2 × 50 ml) and brine, dried over anhydrous sodium sulphate and concentrated in vacuo. Trituration with petroleum ether 40–70° afforded the analytically pure title compound in 80% yield. Rf: 0.69 (AcOEt/hexane 1:1); m.p. 84–85 °C; ^{1}H-NMR ($CDCl_3$): δ 1.24 (t, 3H, $-CH_3$); 3.21 (m, 2H, $-CH_2-CH<$); 4.18 (q, 2H, $-CH_2-O-$); 4.97 (m, 1H, >CH–); 5.45 (b, 2H, $-NH_2$); 6.52 (d, 1H, –NH–); 6.61–7.28 (m, 9H, Ar).^{13}C-NMR ($CDCl_3$): δ 14.19,

38.05, 53.20, 61.63, 115.41, 116.69, 117.29, 127.15, 127.43, 128.60, 129.43, 132.59, 136.04, 148.84, 168.66, 171.75.

*7.1.2. General coupling procedures of anthranoyl-DL-phenylalanine ethyl ester. (Compounds **1a, 4a, 6a, 8a, 9a, 10a–21a**)*

To a suspension of 4.00 mmol of the corresponding acid (except for compounds **9a** and **17a** for which the commercially available acyl chlorides were used) in 30 ml of dry CH_2Cl_2 and cooled in an ice-bath were added portionwise, over a period of 0.5 h, 6.00 mmol (1.25 g) of PCl_5. After the mixture turned into a clear solution, stirring was continued at room temperature for 2 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 1.00 g (3.20 mmol) of **24** in 4 ml of pyridine. After the addition was completed, the reaction mixture was stirred at ambient temperature overnight. Then 150 ml of CH_2Cl_2 were added and the organic layer was washed twice with 40 ml of 1 N HCl, H_2O, 0.1 N NaOH and brine. After drying over Na_2SO_4, the organic phase was rotary evaporated and the residue was purified as described to yield the titled compounds.

*7.1.2.1. 2(*R,S*)-{2-[(1-Methyl-1*H*-indole-2-carbonyl)-amino]benzoylamino}-3-phenyl-propionic acid ethyl ester (**1a**).* Crystallization from MeOH afforded the titled compound in 42% yield. Molecular formula: $C_{28}H_{27}N_3O_4$; TLC (AcOEt/hexane 1:1)—Rf: 0.78; m.p. 153–155 °C; ^{1}H-NMR ($CDCl_3$) δ 1.30 (t, 3H, –CH_3); 3.27 (m, 2H, –CH_2–CH<); 4.14 (s, 3H, >N–CH_3); 4.24 (q, 2H, –O–CH_2); 5.09 (m, 1H, –CH<); 6.72 (d, 1H, –NH–CH<); 7.05–8.74 (m, 14H, Ar); 11.93 (s, 1H, –NH–); ^{13}C-NMR ($CDCl_3$) δ 14.21, 31.79, 37.99, 53.54, 61.92, 105.67, 110.13, 119.71, 120.53, 121.31, 122.35, 122.81, 124.41, 126.19, 126.77, 127.35, 128.68, 129.40, 132.09, 133.05, 135.63, 139.20, 140.04, 160.91, 168.48, 171.26.

*7.1.2.2. 2(*R,S*)-{2-[(Benzo[b]thiophene-2-carbonyl)-amino]-benzoylamino}-3-phenyl-propionic acid ethyl ester (**4a**).* Crystallization from EtOH 95% afforded the titled compound in 63% yield. Molecular formula: $C_{27}H_{24}N_2O_4S$; TLC (AcOEt/hexane 1:1)—Rf: 0.74; m.p. 120–122 °C; ^{1}H-NMR ($CDCl_3$) δ 1.30 (t, 3H, –CH_3); 3.29 (m, 2H, –CH_2–CH<); 4.25 (q, 2H, –O–CH_2); 5.11 (m, 1H, –CH<); 6.86 (d, 1H, –NH–CH<); 7.05–8.75 (m, 14H, Ar); 12.14 (s, 1H, –NH–); ^{13}C-NMR ($CDCl_3$) δ 14.24, 38.04, 53.66, 61.98, 119.75, 121.42, 122.78, 122.95, 123.15, 124.89, 125.40, 125.45, 126.44, 126.78, 127.37, 128.12, 128.70, 129.41, 133.24, 136.45, 138.83, 139.33, 139.57, 142.46, 161.68, 168.48, 171.95.

*7.1.2.3. 2(*R,S*)-{2-[(3-Methyl-1*H*-indene-2-carbonyl)-amino]-benzoylamino}-3-phenyl-propionic acid ethyl ester (**6a**).* Crystallization from MeOH afforded the titled compound in 65% yield. Molecular formula: $C_{29}H_{28}N_2O_4$; TLC (AcOEt/hexane 1:1)—Rf: 0.65; m.p. 138–140 °C; ^{1}H-NMR ($CDCl_3$) δ 1.30 (t, 3H, –CH_3); 2.65 (s, 3H, –CH_3 ind); 3.25 (m, 2H, –CH_2–CH<); 3.76 (s, 2H, –CH_2– ind); 4.24 (q, 2H, –O–CH_2); 5.06 (m, 1H, –CH<); 6.70 (d, 1H, –NH–CH<); 7.03–8.77 (m, 13H, Ar); 11.32 (s, 1H, –NH–); ^{13}C-NMR ($CDCl_3$) δ 12.43, 14.22, 38.13, 38.77, 53.57, 61.90, 119.89, 120.86, 121.63, 122.69, 124.22, 126.66, 126.75, 126.98, 127.30, 127.42, 128.65, 128.83, 129.39, 132.70, 132.94, 135.66, 140.08, 142.48, 145.82, 149.70, 164.60, 168.57, 171.24.

*7.1.2.4. 2(*R,S*)-{2-[(2-Nitrocinnamoyl)-amino]-benzoylamino}-3-phenyl-propionic acid ethyl ester (**8a**).* The title compound was purified by trituration with petroleum ether 40–70° and flash chromatography (silica gel, CH_2Cl_2/AcOEt 4:1); yield 26%; molecular formula: $C_{27}H_{25}N_3O_6$; TLC (AcOEt/hexane 1:1)—Rf: 0.51; m.p. 135–136 °C; ^{1}H-NMR (DMSO-d_6) δ 1.11 (t, 3H, –CH_3); 3.16 (m, 2H, –CH_2–CH<); 4.09 (m, 2H, –CH_2–O–); 4.90 (m, 1H, –CH<); 6.86 (d, 1H, =CH–CO–; J = 15 Hz); 7.13–8.13 (m, 14H, Ar and =CH–C_6H_5); ^{13}C-NMR (DMSO-d_6) δ 14.76, 60.31, 61.26, 119.41, 124.47, 125.54, 126.46, 127.05, 127.19, 128.68, 129.52, 129.76, 130.05, 130.16, 131.52, 134.61, 135.44, 138.45, 142.35, 147.44, 148.90, 154.35, 171.68.

*7.1.2.5. 3-Phenyl-2(*R,S*)-{2-(cinnamoylamino)-benzoylamino}-propionic acid ethyl ester (**9a**).* Crystallization from MeOH afforded the titled compound in 61% yield; molecular formula: $C_{27}H_{26}N_2O_4$; TLC (AcOEt/hexane 1:1)—Rf: 0.74; m.p. 158–160 °C; ^{1}H-NMR ($CDCl_3$) δ 1.30 (t, 3H, –CH_3); 3.26 (m, 2H, –CH_2–CH <); 4.25 (m, 2H, –CH_2–O–); 5.04 (m, 1H, –CH<); 6.57 (d, 1H, =CH–Ph J = 15 Hz); 6.79 (d,1H, –NH–); 7.02–7.59 (m, 13H, Ar); 7.73 (d, 1H, =CH–CO–; J = 15 Hz); 8.75 (d, 1H, Ar); 11.18 (s,1H, –NH–); ^{13}C-NMR ($CDCl_3$) δ 14.22, 38.00, 53.66, 61.95, 119,30, 121.64, 122.11, 122.92, 126.71, 127.38, 128.10, 128.70, 128.86, 129.39, 129.92, 133.03, 134.80, 135.66, 139.96, 141.98, 162.83, 168.87, 170.56.

*7.1.2.6. 3-Phenyl-2(*R,S*)-{2-[(quinoline-2-carbonyl)-amino]-benzoylamino}-propionic acid ethyl ester (**10a**).* Trituration with petroleum ether 40–70° and flash chromatography (silica gel, CH_2Cl_2/AcOEt 4:1) afforded the titled compound in 59% yield. Molecular formula: $C_{28}H_{25}N_3O_4$; TLC (AcOEt/hexane 1:1)–Rf: 0.52; m.p. 153–154 °C; ^{1}H-NMR (DMSO-d_6) δ 1.06 (t, 3H, –CH_3); 3.21 (m, 2H, –CH_2–CH<); 4.07 (q, 2H, –CH_2–O–); 4.74 (m, 1H, –CH<); 7.06–8.74 (m, 15H, Ar); 9.18 (d, 1H, –NH–CH<); 12.78 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 14.65, 36.82, 55.13, 61.39, 119.21, 120.89, 122.70, 123.78, 127.14, 128.86, 129.15, 129.23, 129.68, 129.76, 130.00, 131.43, 132.78, 138.25, 138.54, 139.02, 146.43, 150.17, 163.16, 168.95, 172.06.

*7.1.2.7. 3-Phenyl-2(*R,S*)-{2-[(quinoline-3-carbonyl)-amino]-benzoylamino}-propionic acid ethyl ester (**11a**).* Trituration with petroleum ether 40–70° and flash chromatography (silica gel, CH_2Cl_2/AcOEt 4:1) afforded the titled com-

pound in 46% yield. Molecular formula: $C_{28}H_{25}N_3O_4$; TLC (AcOEt/hexane 1:1)—Rf: 0.49; m.p. 148–149 °C; ^{1}H-NMR (DMSO-d_6) δ 1.09 (t, 3H, –CH_3); 3.16 (m, 2H, –CH_2–CH<); 4.08 (q, 2H, –CH_2–O–); 4.74 (m, 1H, –CH<); 7.06–9.33 (m, 16H, Ar and –NH–CH<); 12.16 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 14.74, 36.91, 55.08, 61.51, 121.53, 121.61, 124.14, 127.19, 127.88, 128.48, 128.87, 129.14, 129.53, 129.80, 130.02, 132.47, 133.22, 136.44, 138.10,139.28, 148.76, 149.36, 163.67, 169.39, 171.83.

*7.1.2.8. 3-Phenyl-2(*R,S*)-{2-[(quinoline-4-carbonyl)-amino]-benzoylamino}-propionic acid ethyl ester (**12a**).* Trituration with petroleum ether 40–70° and flash chromatography (silica gel, CH_2Cl_2/AcOEt 4:1) afforded the titled compound in 14% yield. Molecular formula: $C_{28}H_{25}N_3O_4$; TLC (AcOEt/hexane 1:1)—Rf: 0.30; m.p. 107–108 °C; ^{1}H-NMR (DMSO-d_6) δ 1.02 (t, 3H, –CH_3); 3.11 (m, 2H, –CH_2–CH<); 4.01 (q, 2H, –CH_2–O–); 4.61 (m, 1H, –CH<); 7.07–9.08 (m, 15H, Ar); 9.22 (d, 1H, –NH–CH<); 11.64 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 14.60, 36.87, 54.94, 61.51, 119.45, 122.12, 122.69, 124.36, 124.77, 125.87, 127.19, 128.54, 128.87, 129.07, 129.72, 130.06, 130.95, 133.06, 137.95, 138.41, 141.98, 148.66, 151.07, 165.41, 168.95, 171.80.

*7.1.2.9. 2(*R,S*)-{2-[(Isoquinoline-1-carbonyl)-amino]-benzoylamino}-3-phenyl-propionic acid ethyl ester (**13a**).* Trituration with petroleum ether 40–70° and flash chromatography (silica gel, CH_2Cl_2/AcOEt 4:1) afforded the titled compound in 55% yield. Molecular formula: $C_{28}H_{25}N_3O_4$; TLC (AcOEt/hexane 1:1)—Rf: 0.68; m.p. 171–172 °C; ^{1}H-NMR (DMSO-d_6) δ 1.06 (t, 3H, –CH_3); 3.15 (m, 2H, –CH_2–CH<); 4.06 (q, 2H, –CH_2–O–); 4.67 (m, 1H, –CH<); 7.01–9.16 (m, 15H, Ar); 9.33 (d, 1H, –NH–CH<); 12.49 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 14.68, 36.88, 54.93, 61.42, 121.17, 122.68, 123.81, 125.47, 126.80, 127.08, 127.20 128.13, 128.82, 129.11, 129.68, 129.75, 131.49, 132.75, 137.75, 138.19, 138.71, 141.36, 148.94, 164.76, 168.80, 171.92.

*7.1.2.10. 2(*R,S*)-{2-[(1-Methyl-1*H*-pyrrole-2-carbonyl)-amino]-benzoylamino}-3-phenyl-propionic acid ethyl ester (**14a**).* Trituration with petroleum ether 40–70° followed by flash chromatography (silica gel, AcOEt/CH_2Cl_2 1:4) afforded the titled compound in 19% yield. Molecular formula: $C_{24}H_{25}N_3O_4$; TLC (AcOEt/hexane 1:1)—Rf: 0.67; m.p. 117–118 °C; ^{1}H-NMR (DMSO-d_6) δ 1.13 (t, 3H, –CH_3); 3.16 (m, 2H, –CH_2–CH<); 3.89 (s, 3H, –N–CH_3); 4.10 (q, 2H, –O–CH_2); 4.69 (m, 1H, –CH<); 6.11-8.55 (m, 12H, Ar); 9.20 (d, 1H, –NH–CH<); 11.64 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 14.78, 36.78, 37.12, 55.08, 61.49, 108.07, 113.50, 119.89, 120.34, 122.66, 126.05, 127.23, 128.93, 129.07, 129.76, 130.25, 133.14, 138.18, 140.26, 159.77, 169.62, 171.91.

*7.1.2.11. 3-Phenyl-2(*R,S*)-{2-[(1*H*-pyrrole-2-carbonyl)-amino]-benzoylamino}-propionic acid ethyl ester (**15a**).* Trituration with petroleum ether 40–70° followed by flash chromatography (silica gel, AcOEt/CH_2Cl_2 1:4) afforded the titled compound in 86% yield. Molecular formula: $C_{23}H_{23}N_3O_4$; TLC (AcOEt/hexane 1:1)—Rf: 0.57; m.p. 158 °C; ^{1}H-NMR (DMSO-d_6) δ 1.13 (t, 3H, –CH_3); 3.17 (m, 2H, –CH_2–CH<); 4.11 (q, 2H, –O–CH_2); 4.70 (m, 1H, –CH<); 6.19-8.58 (m, 12H, Ar); 9.22 (d, 1H, –NH–CH<); 11.72 (s, 1H, –NH–); 11.86 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 29.13, 40.22, 47.45, 53.85, 102.38, 103.30, 112.02, 112.68, 115.00, 116.42, 119.16, 119.59, 121.28, 121.42, 122.11, 125.57, 130.51, 132.63, 151.54, 161.96, 164.26.

*7.1.2.12. 2(*R,S*)-{2-[(Furan-2-carbonyl)-amino]-benzoylamino}-3-phenyl-propionic acid ethyl ester (**16a**).* Trituration with petroleum ether 40–70° followed by flash chromatography (silica gel, AcOEt/CH_2Cl_2 1:4) afforded the titled compound in 50% yield. Molecular formula: $C_{23}H_{22}N_2O_5$; TLC (AcOEt/hexane 1:1)—Rf: 0.47; m.p. 118 °C; ^{1}H-NMR (DMSO-d_6) δ 1.12 (t, 3H, –CH_3); 3.16 (m, 2H, –CH_2–CH<); 4.11 (q, 2H, –O–CH_2); 4.71 (m, 1H, –CH<); 6.72–8.55 (m, 12H, Ar); 9.22 (d, 1H, –NH–CH<); 11.89 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 14.71, 36.83, 54.99, 61.53, 113.38, 116.06, 120.70, 120.99, 123.63, 127.19, 128.89, 129.06, 129.78, 133.16, 138.17, 139.11, 146.78, 148.05, 156.20, 169.18, 171.86.

*7.1.2.13. 3-Phenyl-2(*R,S*)-{2-[(thiophene-2-carbonyl)-amino]-benzoylamino}-propionic acid ethyl ester (**17a**).* Trituration with petroleum ether 40–70° followed by flash chromatography (silica gel, AcOEt/CH_2Cl_2 1:4) afforded the titled compound in 77% yield. Molecular formula: $C_{23}H_{22}N_2O_4S$; TLC (AcOEt/hexane 1:1)—Rf: 0.71; m.p. 154–155 °C; ^{1}H-NMR (DMSO-d_6) δ 1.13 (t, 3H, –CH_3); 3.17 (m, 2H, –CH_2–CH<); 4.11 (q, 2H, –O–CH_2); 4.72 (m, 1H, –CH<); 7.12–8.50 (m, 12H, Ar); 9.27 (d, 1H, –NH–CH<); 12.02 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 14.78, 36.84, 55.08, 61.54, 120.46, 120.90, 123.60, 127.22, 128.92, 129.10, 129.18, 129.78, 133.14, 133.29, 138.15, 139.51, 140.27,159.85, 169.49, 171.81.

*7.1.2.14. 3-Phenyl-2(*R,S*)-{2-[(pyridine-2-carbonyl)-amino]-benzoylamino}-propionic acid ethyl ester (**18a**).* Trituration with petroleum ether 40–70° and flash chromatography (silica gel, CH_2Cl_2/AcOEt 4:1) afforded the titled compound in 71% yield. Molecular formula: $C_{24}H_{23}N_3O_4$; TLC (AcOEt/hexane 1:1)—Rf: 0.41; m.p. 138–139 °C; ^{1}H-NMR (DMSO-d_6) δ 1.09 (t, 3H, –CH_3); 3.17 (m, 2H, –CH_2–CH<); 4.09 (q, 2H, –CH_2–O–); 4.68 (m, 1H, –CH<); 7.09–8.74 (m, 13H, Ar); 9.15 (d, 1H, –NH–CH<); 12.48 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6)) δ 14.71, 36.86, 54.94, 61.46, 121.28, 122.58, 123.04, 123.80, 127.17, 127.80, 128.88, 129.07, 129.76, 132.72, 138.21, 138.54, 138.81, 149.38, 150.19, 163.14, 168.74, 171.98.

*7.1.2.15. 3-Phenyl-2(*R,S*)-{2-[(pyridine-3-carbonyl)-amino]-benzoylamino}-propionic acid ethyl ester (**19a**).* Trituration with petroleum ether 40–70° and flash chromatogra-

phy (silica gel, CH_2Cl_2/AcOEt 4:1) afforded the titled compound in 70% yield. Molecular formula: $C_{24}H_{23}N_3O_4$; TLC (AcOEt/hexane 1:1)—Rf: 0.23; m.p. 134–135 °C; ^{1}H-NMR (DMSO-d_6) δ 1.12 (t, 3H, –CH_3); 3.17 (m, 2H, –CH_2–CH<); 4.10 (q, 2H, –CH_2–O–); 4.74 (m, 1H, –CH<); 7.10–9.03 (m, 13H, Ar); 9.30 (d, 1H, –NH–CH<); 12.06 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 14.74, 36.89, 55.08, 61.52, 121.21, 121.33, 124.04, 124.65, 127.19, 128.88, 129.11, 129.80, 130.67, 133.24, 135.43, 138.11, 139.30, 148.69, 153.32, 163.56, 169.42, 171.81.

*7.1.2.16. 3-Phenyl-2(*R,S*)-{2-[(pyridine-4-carbonyl)-amino]-benzoylamino}-propionic acid ethyl ester (**20a**).* Trituration with petroleum ether 40–70° and flash chromatography (silica gel, CH_2Cl_2/AcOEt 4:1) afforded the titled compound in 67% yield. Molecular formula: $C_{24}H_{23}N_3O_4$; TLC (AcOEt/hexane 1:1)—Rf: 0.24; m.p. 127–128 °C; ^{1}H-NMR (DMSO-d_6) δ 1.12 (t, 3H, –CH_3); 3.17 (m, 2H, –CH_2–CH<); 4.11 (q, 2H, –CH_2–O–); 4.75 (m, 1H, –CH <); 7.10–8.86 (m, 13H, Ar); 9.34 (d, 1H, –NH–CH<); 12.16 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 14.76, 36.89, 55.05, 61.56, 121.14, 121.31, 121.41, 124.27, 127.21, 128.90, 129.15, 129.82, 133.31, 138.11 139.10, 141.97, 151.48, 163.38, 169.34, 171.79.

*7.1.2.17. 3-Phenyl-2(*R,S*)-{2-[(pyrazine-2-carbonyl)-amino]-benzoylamino}-propionic acid ethyl ester (**21a**).* Trituration with petroleum ether 40–70° and flash chromatography (silica gel, CH_2Cl_2/AcOEt 4:1) afforded the titled compound in 49% yield. Molecular formula: $C_{23}H_{22}N_4O_4$; TLC (AcOEt/hexane 1:1)—Rf: 0.36; m.p. 152–153 °C; ^{1}H-NMR (DMSO-d_6) δ 1.10 (t, 3H, –CH_3); 3.16 (m, 2H, –CH_2–CH<); 4.10 (q, 2H, –CH_2–O–); 4.70 (m, 1H, –CH<); 7.07–9.33 (m, 13H, Ar and –NH–CH<); 12.52 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 14.74, 36.89, 54.94, 61.49, 121.29, 122.16, 124.16, 127.15, 128.86, 129.12, 129.77, 132.93, 138.18, 138041, 144.18, 144.61, 145.02, 148.70, 162.04, 168.73, 171.91.

*7.1.3. 2(*R,S*)-{2-[(1*H*-Benzoimidazole-2-carbonyl)-amino]-benzoylamino}-3-phenyl-propionic acid ethyl ester (**2a**)*

To a solution of compound **24** (0.62 g, 2.0 mmol) and triethylamine (0.28 ml, 2.0 mmol) in 15 ml of absolute ethanol was slowly added 2-trichloromethylbenzimidazole (0.47 g, 2.0 mmol). The solution was stirred at room temperature for 24 h. The reaction mixture was filtered and the collected solid was washed with ethanol several times to afford 0.11 g (12%) of compound **2a**. Molecular formula: $C_{26}H_{24}N_4O_4$; TLC (AcOEt/hexane 1:1)—Rf: 0.48; m.p. 244–245 °C; ^{1}H-NMR (DMSO-d_6) δ 1.08 (t, 3H, –CH_3); 3.14 (m, 2H, –CH_2–CH<); 4.07 (q, 2H, –O–CH_2); 4.70 (m, 1H, –CH<); 7.10–8.63 (m, 13H, Ar); 9.15 (d, 1H, –NH–CH<); 12.36 (s, 1H, –NH–); 13.47 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 14.74, 36.90, 55.00, 61.57, 113.38, 121.28, 121.57, 123.70, 124.08, 125.20, 127.20, 128.93 129.18, 129.79, 133.08, 135.58, 138.17, 138.56, 143.10, 145,93, 157.79, 168.75, 171.99.

*7.1.4. 2(*R,S*)-{2-[(Benzofuran-2-carbonyl)-amino]-benzoylamino}-3-phenyl-propionic acid ethyl ester (**3a**)*

Benzofuran-2-carboxylic acid (0.81 g, 5.0 mmol) was dissolved in 50 ml of dry tetrahydrofuran and treated with *N,N*'-carbonyldiimidazole (0.81 g, 5.0 mmol). The reaction mixture, protected from moisture, was stirred at room temperature for 1 h. Then a solution of **24** (1.56 g, 5.0 mmol) in 50 ml of dry tetrahydrofuran was added. The reaction mixture was kept at room temperature for 48 h and the solvent was evaporated. Trituration of the residue with hot methanol afforded compound **3a** analytically pure in 54% yield. Molecular formula: $C_{27}H_{24}N_2O_5$; TLC (AcOEt/hexane 1:1)—Rf: 0.69; m.p. 162–164 °C; ^{1}H-NMR ($CDCl_3$) δ 1.29 (t, 3H, –CH_3); 3.30 (m, 2H, –CH_2–CH<); 4.24 (q, 2H, –O–CH_2); 5.13 (m, 1H, –CH<); 6.82 (d, 1H, –NH–CH<); 7.07-8.77 (m, 14H, Ar); 12.08 (s, 1H, –NH–); ^{13}C-NMR ($CDCl_3$) δ 14.21, 38.02, 53.63, 61.90, 111.11, 112.40, 120.36, 121.80, 122.64, 123.41, 123.72, 126.81, 127.06, 127.30, 127.65, 128.65, 129.40, 132.99, 135.72, 139.15, 149.06, 157.25, 168.26, 171.30.

*7.1.5. 2(*R,S*)-{2-[(1*H*-Indene-2-carbonyl)-amino]-benzoylamino}-3-phenyl-propionic acid ethyl ester (**5a**)*

Oxalyl dibromide 21.5 mmol (2.02 ml) was added dropwise to 43.0 mmol (5.0 g) of indene. The reaction mixture was stirred under reflux for 4 h and then was quenched with ice-water. A solution of 10% Na_2CO_3 was added and the aqueous layer was washed with ether. The aqueous layer was then acidified with 1 N HCl and extracted twice with ether. The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure to afford 1.92 g (56%) of indene-2-carboxylic acid which was used in the next step without further purification. m.p.: 236 °C dec. (Lit. 232–233 °C [18]); TLC: R_f = 0.76 (AcOEt/MeOH 2:1); ^{1}H-NMR ($CDCl_3$): δ 3.64 (s, 2H, –CH_2–); 7.33–7.70 (m, 5H, Ar and –CH=); 12.52 (b, 1H, –OH). ^{13}C-NMR ($CDCl_3$): δ 38.86, 124.00, 125.01, 127.44, 127.96, 139.09, 140.82, 143.26, 145.30, 166.35. To a solution of compound **24** (1.50 g, 4.8 mmol) and triethylamine (0.8 ml, 5.8 mmol) in 15 ml of dry CH_2Cl_2 was added 2-chloro-1-methylpyridinium iodide (2.0 g, 7.83 mmol) and indene-2-carboxylic acid (0.93 g, 5.8 mmol) and the mixture was stirred under reflux overnight. After addition of water (20 ml) the reaction mixture was extracted with dichloromethane (3 × 20 ml). The combined organic layers were washed with 1 N HCl, water, 1 N NaOH and brine. The dried organic phase was rotary evaporated and the residue was chromatographed on silica gel eluting with CH_2Cl_2:AcOEt (4:1) to afford 0.22 g (10%) of compound **5a**. Molecular formula: $C_{28}H_{26}N_2O_4$; TLC (AcOEt/hexane 1:1)—Rf: 0.65; m.p. 146 °C; ^{1}H-NMR (DMSO-d_6) δ 1.16 (t, 3H, –CH_3); 3.17 (m, 2H, –CH_2–CH<); 3.70 (s, 2H, –CH_2– ind); 4.13 (q, 2H, –O–CH_2); 4.76 (m, 1H, –CH<); 7.13–8.60 (m, 14H, Ar and –CH=); 9.25 (d, 1H, –NH–CH<); 11.68 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 14.81, 36.91, 38.41, 55.08, 61.54, 120.40, 120.75, 123.29, 123.84, 125.00, 127.19, 127.62, 127.90, 128.89, 129.06,

129.82, 133.17, 137.17, 138.18, 139.78, 142.51, 143.24, 144.58, 162.56, 169.52, 171.90.

*7.1.6. 2(*R,S*)-{2-[(2-Acetylamino-cinnamoyl)-amino]-benzoylamino}-3-phenyl-propionic acid ethyl ester (**7a**)*

A solution of 2-acetylamino-cinnamic acid (1.00 g, 4.87 mmol) and triethylamine (0.69 ml, 4.87 mmol) in 25 ml of dry dichloromethane cooled to –10 °C was treated with isobutyl chloroformate (0.63 ml, 4.87 mmol). The resulting mixture was stirred at –10 °C for 20 min and treated dropwise with a solution of compound **24** (1.52 g, 4.87 mmol) in 25 ml of dry dichloromethane. After the addition was complete, the reaction was stirred at room temperature for 12 h. The solvents were evaporated, the residue was dissolved in dichloromethane, the organic layer was washed with diluted aqueous sodium hydroxide solution and with water, dried (sodium sulphate), and evaporated to dryness. The residue was purified by trituration with petroleum ether 40–70° to give the title compound in 17% yield. Molecular formula $C_{29}H_{29}N_3O_5$; TLC (AcOEt/hexane 1:1)—Rf: 0.25; m.p. 164–165 °C; ^{1}H-NMR ($CDCl_3$) δ 1.27 (t, 3H, –CH_3); 2.25 (s, 3H, –CO–CH_3); 3.21 (m, 2H, –CH_2–CH<); 4.21 (q, 2H, –CH_2–O–); 4.92 (m, 1H, –CH<); 6.74 (d, 1H, –NH–CH<); 7.00–7.46 (m, 14H, Ar and –CH=); 8.72 (d, 1H, Ar); 11.79 (s, 1H, –NH–); 12.08 (s, 1H, –NH–); ^{13}C-NMR ($CDCl_3$) δ 14.16, 23.37, 37.87, 53.63, 61.83, 119.64, 121.27, 122.91, 126.57, 127.15, 128.57, 128.85, 129.07, 129.30, 131.01, 132.95, 133.76, 135.62, 139.57, 163.10, 168.23, 169.87, 171.05.

*7.1.7. 3-Phenyl-2-{2-[(4,5,6,7-tetrahydro-1*H*-indole-2-carbonyl)-amino]-benzoylamino}-propionic acid ethyl ester (**22a**)*

A mixture of 0.658 g (15.68 mmol) of LiOH·H_2O and 15 ml of H_2O was added to a solution of 0.758 g (3.92 mmol) of 4,5,6,7-tetrahydro-1*H*-indole-2-carboxylic acid ethyl ester in 30 ml of EtOH 95%. The reaction was stirred under reflux for 6 h (TLC monitoring), and then was concentrated in vacuo. The residue was diluted with 100 ml of H_2O and washed with AcOEt (1 × 30 ml). The aqueous phase was acidified and the filtered solid (0.525 g, 81%) was used in the next step without further purification.

Thionyl chloride (0.25 ml) (0.408 g, 3.43 mmol) were added dropwise to a solution of 0.525 g (3.18 mmol) of 4,5,6,7-tetrahydro-1*H*-indole-2-carboxylic acid in 10 ml of anhydrous pyridine. The reaction mixture was stirred at room temperature under argon and three drops of DMF were added. The conversion into chloride was complete in 1 h (TLC monitoring). 1.00 g (3.18 mmol) of **24** was then added and the mixture was stirred for 12 h under argon. The mixture was diluted with CH_2Cl_2 (50 ml) and washed with H_2O (2 × 25 ml) and brine (1 × 25 ml). The organic phase was dried over Na_2SO_4, filtered and evaporated. The residue was chromatographed on silica gel (AcOEt–CH_2Cl_2 5:95), and tritured with cold diethyl ether, to afford 0.573 g (39%) of a white solid. Molecular formula $C_{27}H_{29}N_3O_4$; TLC (AcOEt/hexane 1:1)—Rf: 0.55; m.p. 207 °C; ^{1}H-NMR (DMSO-d_6) δ 1.14 (t, 3H, –CH_3); 1.69 (m, 4H, –CH_2–); 2.50 (m, 4H, –CH_2–); 3.16 (m, 2H, –CH_2–CH<); 4.11 (q, 2H, –CH_2–O–); 4.69 (m, 1H, –CH<); 6.35 (s, 1H, Ar); 7.06–7.31 (m, 6H, Ar); 7.49 (t, 1H, Ar); 7.69 (d, 1H, Ar); 8.59 (d, 1H, Ar); 9.18 (d, 1H, –NH–CH<); 11.34 (s, 1H, –NH–); 11.55 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 13.99, 22.29, 22.46, 22.59, 23.16, 35.94, 54.31, 60.66, 108.98, 117.71, 118.47, 119.24, 121.37, 123.72, 126.43, 128.11, 128.24, 128.96, 132.37, 132.99, 137.37, 139.85, 158.52, 168.85, 171.16.

*7.1.8. 3-Phenyl-2-{2-[(1,4,5,6-tetrahydro-cyclopenta[b]-pyrrole-2-carbonyl)-amino]-benzoylamino}-propionic acid ethyl ester (**23a**)*

23a was synthesized and purified as described under Section 7.1.7. The hydrolysis of 0.686 g (3.83 mmol) of tetrahydro-cyclopenta[*b*]pyrrole-2-carboxylic acid ethyl ester afforded 0.481 g (3.18 mmol, 83%) of the corresponding acid, which was used without purification in the next step to give 0.350 g (25%) of the titled compound as a white solid. Molecular formula $C_{26}H_{27}N_3O_4$; TLC (AcOEt/hexane 1:1)—Rf: 0.52; m.p. 208–209 °C dec.; ^{1}H-NMR (DMSO-d_6) δ 1.14 (t, 3H, –CH_3); 2.33 (m, 2H, –CH_2–); 2.63 (m, 4H, –CH_2–); 3.17 (m, 2H, –CH_2–CH<); 4.11 (q, 2H, –CH_2–O–); 4.69 (m, 1H, –CH<); 6.38 (s, 1H, Ar); 7.06–7.40 (m, 6H, Ar); 7.51 (t, 1H, Ar); 7.70 (d, 1H, Ar); 8.57 (d, 1H, Ar); 9.18 (d, 1H, –NH–CH<); 11.48 (s, 1H, –NH–); 11.59 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 13.98, 24.37, 24.65, 28.58, 35.94, 54.29, 60.67, 105.46, 118.43, 119.25, 121.37, 126.43, 127.10, 128.11, 128.23, 128.58, 128.94, 132.37, 137.37, 139.87, 142.57, 158.49, 168.85, 171.14.

*7.1.9. General procedure for the synthesis of compounds **1–23***

A mixture of 5.0 mmol of the corresponding ethyl ester (compounds **1a–23a**) in water (25 ml) and tetrahydrofuran (25 ml) and in the presence of sodium hydroxide (0.20 g, 5.0 mmol) was kept at room temperature for 4 h. The organic solvent was removed under reduced pressure and the aqueous solution was cooled in an ice bath. After cooling, the aqueous solution was adjusted to pH 2–3 (except for compounds **2** and **5** where the pH was adjusted to 4–5) with diluted HCl to obtain the precipitation of the corresponding acid.

*7.1.9.1. 2(*R,S*)-{2-[(1-Methyl-1*H*-indole-2-carbonyl)-amino]-benzoylamino}-3-phenyl-propionic acid (**1**).* Crystallization from EtOH 75% afforded the titled compound in 60% yield. TLC (AcOEt/MeOH 2:1)—Rf: 0.50; m.p. 186–188 °C; ^{1}H-NMR (DMSO-d_6) δ 3.28 (m, 2H, –CH_2–CH<); 4.05 (s, 3H, –CH_3); 4.76 (m, 1H, –CH<); 7.02–8.60 (m, 14H, Ar); 9.18 (d, 1H, –NH–CH<); 12.12 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 31.32, 35.97, 53.92, 104.46, 110.51, 119.47, 119.76, 120.37, 121.75, 122.47, 124.11, 125.25, 126.22, 127.98, 128.19, 128.86, 131.73, 132.29, 137.78, 138.83, 138.93, 169.59, 168.50, 172.51. MS (ES) *m/z* 442 $[MH]^+$; MW: 441 (calcd. for $C_{26}H_{23}N_3O_4$).

*7.1.9.2. 2(*R,S*)-{2-[(1*H*-Benzoimidazole-2-carbonyl)-amino]-benzoylamino}-3-phenyl-propionic acid (**2**).* The collected precipitate was analytically pure. TLC (AcOEt/MeOH 2:1)—Rf: 0.62; m.p. 271–272 °C; ^{1}H-NMR (DMSO-d_6) δ 3.10 (m, 2H, –CH_2–); 4.73 (m, 1H, –CH<); 7.07–8.65 (m, 13H, Ar); 9.02 (d, 1H, –NH–CH<); 12.56 (s, 1H, –NH–); 13.46 (s, 1H, –NH); ^{13}C-NMR (DMSO-d_6) δ 36.98, 54.73, 113.35, 121.26,123.58, 124.00, 125.26, 127.06, 128.87, 129.15, 129.73, 133.06, 135.54, 138.65, 138.81, 143.16, 145.99, 157.82, 168.55, 173.51. MS (ES) *m/z* 429 $[MH]^+$; MW: 428 (calcd. for $C_{24}H_{20}N_4O_4$).

*7.1.9.3. 2(*R,S*)-{2-[(Benzofuran-2-carbonyl)-amino]-benzoylamino}-3-phenyl-propionic acid (**3**).* Trituration with hot MeOH afforded the titled compound in 52% yield. TLC (AcOEt/MeOH 3:1)—Rf: 0.25; m.p. 256–257 °C; ^{1}H-NMR (DMSO-d_6) δ 3.30 m, 2H, –CH_2–CH<); 4.78 (m, 1H, –CH<); 7.03–8.58 (m, 14H, Ar); 9.16 (d, 1H, –NH–CH<); 12.24 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 36.09, 53.80, 110.88, 111.85, 120.12, 120.32, 122.82, 123.08, 123.81, 126.13, 126.96, 127.29, 127.91, 128.17, 128.85, 132.22, 137.72, 138.08, 148.32, 154.25, 155.87, 168.13, 172.51. MS (ES) *m/z* 429 $[MH]^+$; MW: 428 (calcd. for $C_{25}H_{20}N_2O_5$).

*7.1.9.4. 2(*R,S*)-{2-[(Benzo[b]thiophene-2-carbonyl)-amino]-benzoylamino}-3-phenyl-propionic acid (**4**).* Crystallization from MeOH afforded the titled compound in 63% yield. TLC (AcOEt/MeOH 3:1)—Rf: 0.33; m.p. 207–209 °C; ^{1}H-NMR (DMSO-d_6) δ 3.28 (m, 2H, –CH_2–CH<); 4.80 (m, 1H, –CH<); 7.09–8.51 (m, 14H, Ar); 9.21 (d, 1H, –NH–CH<); 12.31(s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 36.01, 53.91, 119.65, 120.13, 122.77, 122.94, 125.03, 125.08, 125.45, 126.20, 126.60, 127.96, 128.19, 128.87, 132.38, 137.73, 138.51, 138.70, 139.32, 140.33, 159.41, 168.43, 172.46. MS (ES) *m/z* 445 $[MH]^+$; MW: 444 (calcd. for $C_{25}H_{20}N_2O_4S$).

*7.1.9.5. 2(*R,S*)-{2-[(1*H*-Indene-2-carbonyl)-amino]-benzoylamino}-3-phenyl-propionic acid (**5**).* The collected precipitate was analytically pure. TLC (AcOEt/MeOH 2:1)—Rf: 0.59; m.p. 209–210 °C; ^{1}H-NMR (DMSO-d_6) δ 3.07 (m, 2H, –CH_2–CH<); 3.69 (s, 2H, –CH_2– ind); 4.72 (m, 1H, –CH<); 7.04–8.60 (m, 14H, Ar and –CH=); 9.07 (d, 1H, –NH–CH<); 11.79 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 37.07, 38.40, 55.01, 120.42, 120.65, 123.24, 123.88, 125.03, 126.99, 127.59, 127.86, 128.79, 128.93, 129.80, 133.04, 137.18, 138.81, 139.84, 142.55, 143.26, 144.59, 162.58, 169.22, 173.36. MS (ES) *m/z* 427 $[MH]^+$; MW: 426 (calcd. for $C_{26}H_{22}N_2O_4$).

*7.1.9.6. 2(*R,S*)-{2-[(3-Methyl-1*H*-indene-2-carbonyl)-amino]-benzoylamino}-3-phenyl-propionic acid (6).* Trituration with Et_2O afforded the titled compound in 58% yield. TLC (AcOEt/MeOH 2:1)—Rf: 0.50; m.p. 127–129 °C; ^{1}H-NMR (DMSO-d_6) δ 2.53 (s, 3H, –CH_3); 3.23 (m, 2H, –CH_2–CH<); 3.62 (s, 2H, –CH_2– ind); 4.71 (m, 1H, –CH<); 7.06–8.59 (m, 13H, Ar); 9.11 (d, 1H, –NH–CH<); 11.39 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 11.85, 36.05, 37.46, 53.87, 119.91, 120.00, 120.66, 122.21, 123.78, 126.10, 126.58, 127.25, 127.89, 128.00, 128.87, 131.96, 132.48, 137.80, 138.95, 141.76, 144.95, 148.30, 163.12, 168.47, 172.50. MS (ES) *m/z* 441 $[MH]^+$; MW: 440 (calcd. for $C_{27}H_{24}N_2O_4$).

*7.1.9.7. 2(*R,S*)-{2-[(2-Acetylamino-cinnamoyl)-amino]-benzoylamino}-3-phenyl-propionic acid (**7**).* Purification method: crystallization from AcOEt/Hexane; TLC (AcOEt/MeOH 2:1)—Rf: 0.43; m.p. 126–127 °C; ^{1}H-NMR (DMSO-d_6) δ 2.04 (s, 3H, –CH_3); 3.13 (m, 2H, –CH_2–CH<); 4.59 (m, 1H, –CH<); 7.08–8.65 (m, 15H, Ar and –CH=); 9.02 (d, 1H, –NH–CH<); 9.83 (s, 1H, –NH–); 12.06 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 23.47, 36.84, 54.90, 119.99, 120.28, 123.14, 127.11, 128.94, 129.04, 129.32, 129.65, 129.80, 130.34, 130.61, 130.91, 133.18, 134.37, 138.68, 140.14, 163.92, 169.02, 170.85, 173.31. MS (ES) *m/z* 472 $[MH]^+$; MW: 471 (calcd. for $C_{27}H_{25}N_3O_5$).

*7.1.9.8. 2(*R,S*)-{2-[(2-Nitrocinnamoyl)-amino]-benzoylamino}-3-phenyl-propionic acid (**8**).* Purification method: crystallization from AcOEt/Hexane; TLC (AcOEt/MeOH 2:1)—Rf: 0.47; m.p. 196–198 °C; ^{1}H-NMR (DMSO-d_6) δ 3.20 (m, 2H, –CH_2–CH<); 4.69 (m, 1H, –CH<); 6.85 (d, 1H, =CH–CO– *J*= 15 Hz); 7.14–8.51 (m, 14H, Ar and =CH–C_6H_5); 9.06 (d, 1H, –NH–CH<); 11.19 (s, 1H, –NH–); 13.00 (b, 1H, –OH); ^{13}C-NMR (DMSO-d_6) δ 36.92, 54.87, 121.62, 122.00, 123.86, 125.35, 127.10, 127.67, 128.88, 129.74, 129.89, 130.18, 131.30, 132.68, 134.47, 136.54, 138.66, 139.00, 149.06, 163.32, 168.75, 173.54. MS (ES) *m/z* 460 $[MH]^+$; MW: 459 (calcd. for $C_{25}H_{21}N_3O_6$).

*7.1.9.9. 3-Phenyl-2(*R,S*)-[2-(cinnamoylamino)-benzoylamino]-propionic acid (**9**).* Purification method: crystallization from MeOH; TLC (AcOEt/MeOH 3:1)—Rf: 0.29; m.p. 209–211 °C; ^{1}H-NMR (DMSO-d_6) δ 3.23 (m, 2H, –CH_2–CH<); 4.70 (m, 1H, –CH<); 6.79 (d, 1H, =CH–CO– *J* = 15 Hz); 7.15–8.55 (m, 14H, Ar and =CH–C_6H_5); 9.07 (d, 1H, –NH–CH<); 11.11 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 36.00, 53.91, 120.57, 120.79, 122.17, 122.61, 126.22, 127.98, 128.70, 128.86, 129.77, 131.78, 134.23, 137.76, 138.49, 140.89, 163.29, 168.05, 172.70. MS (ES) *m/z* 415 $[MH]^+$; MW: 414 (calcd. for $C_{25}H_{22}N_2O_4$).

*7.1.9.10. 3-Phenyl-2(*R,S*)-{2-[(quinoline-2-carbonyl)-amino]-benzoylamino}-propionic acid (**10**).* Purification method: crystallization from EtOH 95%; TLC (AcOEt/MeOH 2:1)—Rf: 0.41; m.p. 206–207 °C; ^{1}H-NMR (DMSO-d_6) δ 3.22 (m, 2H, –CH_2–CH<); 4.79 (m, 1H, –CH<); 6.98–8.74 (m, 15H, Ar); 9.03 (d, 1H, –NH–CH<); 12.84 (s, 1H, –NH–); 12.95 (b, 1H, –OH); ^{13}C-NMR (DMSO-d_6) δ 36.96, 54.69, 119.24, 120.84, 122.59, 123.73, 126.97, 128.77, 129.10, 129.22, 129.70, 130.16, 131.47, 132.71, 138.66, 138.70, 138.99, 146.45, 150.24, 163.19, 168.65, 173.56. MS (ES) *m/z* 440 $[MH]^+$; MW: 439 (calcd. for $C_{26}H_{21}N_3O_4$).

*7.1.9.11. 3-Phenyl-2(*R,S*)-{2-[(quinoline-3-carbonyl)-amino]-benzoylamino}-propionic acid (**11**).* Purification method: trituration with hot AcOEt; TLC (AcOEt/MeOH 2:1)—Rf: 0.38; m.p. 267–268 °C; ^{1}H-NMR (DMSO-d_6) δ 3.24 (m, 2H, –CH_2–CH<); 4.76 (m, 1H, –CH<); 7.02–9.28 (m, 16H, Ar and –NH–CH<); 12.25 (s, 1H, –NH–); 12.99 (b, 1H, –OH); ^{13}C-NMR (DMSO-d_6) δ 36.95, 54.93, 121.36, 124.04, 127.05, 127.21, 127.93, 128.44, 128.82, 129.07, 129.51, 129.77, 130.11, 132.45, 133.17, 136.45, 138.62, 139.39, 148.76, 149.36, 163.66, 169.29, 173.34. MS (ES) *m/z* 440 $[MH]^+$; MW: 439 (calcd. for $C_{26}H_{21}N_3O_4$).

*7.1.9.12. 3-Phenyl-2(*R,S*)-{2-[(quinoline-4-carbonyl)-amino]-benzoylamino}-propionic acid (**12**).* Purification method: trituration with hot AcOEt; TLC (AcOEt/MeOH 2:1)—Rf: 0.27; m.p. 245 °C dec.; ^{1}H-NMR (DMSO-d_6) δ 3.15 (m, 2H, –CH_2–CH<); 4.62 (m, 1H, –CH<); 7.04–9.24 (m, 16H, Ar and –NH–CH<); 11.81 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 36.91, 54.81, 119.89, 122.19, 122.79, 124.89, 126.44, 127.04, 127.33, 128.81, 129.12, 129.51, 129.73, 132.51, 132.96, 138.47, 138.57, 144.89, 145.38, 149.51, 164.59, 168.72, 173.32. MS (ES) *m/z* 440 $[MH]^+$; MW: 439 (calcd. for $C_{26}H_{21}N_3O_4$).

*7.1.9.13. 2(*R,S*)-{2-[(Isoquinoline-1-carbonyl)-amino]-benzoylamino}-3-phenyl-propionic acid (**13**).* Purification method: trituration with hot AcOEt; TLC (AcOEt/MeOH 2:1)—Rf: 0.40; m.p. 218–219 °C; ^{1}H-NMR (DMSO-d_6) δ 3.17 (m, 2H, –CH_2–CH<); 4.70 (m, 1H, –CH<); 6.90–9.30 (m, 16H, Ar and –NH–CH<); 12.58 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 37.01, 54.60, 121.09, 122.37, 123.76, 125.42, 126.79, 126.95, 127.22 128.12, 128.75, 129.10, 129.64, 129.71, 131.47, 132.76, 137.73, 138.62, 138.92, 141.44, 149.04, 164.78, 168.60, 173.52. MS (ES) *m/z* 440 $[MH]^+$; MW: 439 (calcd. for $C_{26}H_{21}N_3O_4$).

*7.1.9.14. 2(*R,S*)-{2-[(1-Methyl-1*H*-pyrrole-2-carbonyl)-amino]-benzoylamino}-3-phenyl-propionic acid (**14**).* Trituration with hot AcOEt afforded the titled compound in 72% yield. TLC (AcOEt/MeOH 2:1)—Rf: 0.48; m.p. 201–202 °C; ^{1}H-NMR (DMSO-d_6) δ 3.16 (m, 2H, –CH_2–CH<); 3.89 (s, 3H, –N–CH_3); 4.68 (m, 1H, –CH<); 6.11–8.55 (m, 12H, Ar); 9.08 (d, 1H, –NH–CH<); 11.75 (s, 1H, –NH–); 12.95 (b, 1H, –OH); ^{13}C-NMR (DMSO-d_6) δ 36.81, 37.12, 54.90, 108.12, 113.53, 119.82, 120.21, 122.59, 126.10, 127.10, 128.89, 129.00, 129.72, 130.22, 133.08, 138.70, 140.36, 159.78, 169.44, 173.39. MS (ES) *m/z* 392 $[MH]^+$; MW: 391 (calcd. for $C_{22}H_{21}N_3O_4$).

*7.1.9.15. 3-Phenyl-2(*R,S*)-{2-[(1*H*-pyrrole-2-carbonyl)-amino]-benzoylamino}-propionic acid (**15**).* Trituration with hot AcOEt afforded the titled compound in 80% yield. TLC (AcOEt/MeOH 2:1)—Rf: 0.45; m.p. 267 °C dec.; ^{1}H-NMR (DMSO-d_6) δ 3.23 (m, 2H, –CH_2–CH<); 4.70 (m, 1H, –CH<); 6.21–8.59 (m, 12H, Ar); 9.09 (d, 1H, –NH–CH<); 11.82 (s, 1H, –NH–); 11.85 (s, 1H, –NH–); 12.95 (b, 1H, –OH); ^{13}C-NMR (DMSO-d_6) δ 36.82, 54.91, 110.05, 111.02, 119.63, 120.24, 122.57, 124.04, 126.87, 127.12, 128.90, 129.02, 129.72, 133.15, 138.69, 140.40, 159.21, 169.45, 173.42. MS (ES) *m/z* 378 $[MH]^+$; MW: 377 (calcd. for $C_{21}H_{19}N_3O_4$).

*7.1.9.16. 2(*R,S*)-{2-[(Furan-2-carbonyl)-amino]-benzoylamino}-3-phenyl-propionic acid (**16**).* Crystallization from EtOH 95% afforded the titled compound in 65% yield. TLC (AcOEt/MeOH 2:1)—Rf: 0.41; m.p. 229 °C; ^{1}H-NMR (DMSO-d_6) δ 3.20 (m, 2H, –CH_2–CH<); 4.71 (m, 1H, –CH<); 6.72–8.57 (m, 12H, Ar); 9.09 (d, 1H, –NH–CH<); 12.07 (s, 1H, –NH–); 13.00 (b, 1H, –OH); ^{13}C-NMR (DMSO-d_6) δ 36.93, 54.79, 113.35, 115.06, 120.43, 120.86, 123.55, 127.07, 128.83, 129.01, 129.72, 133.14, 138.64, 139.32, 146.80, 148.08, 156.21, 169.00, 173.41. MS (ES) *m/z* 379 $[MH]^+$; MW: 378 (calcd. for $C_{21}H_{18}N_2O_5$).

*7.1.9.17. 3-Phenyl-2(*R,S*)-{2-[(thiophene-2-carbonyl)-amino]-benzoylamino}-propionic acid (**17**).* Trituration with hot AcOEt afforded the titled compound in 83% yield. TLC (AcOEt/MeOH 2:1)—Rf: 0.43; m.p. 207 °C; ^{1}H-NMR (DMSO-d_6) δ 3.24 (m, 2H, –CH_2–CH<); 4.73 (m, 1H, –CH<); 7.09–8.53 (m, 12H, Ar); 9.18 (d, 1H, –NH–CH<); 12.16 (s, 1H, –NH–); 12.95 (b, 1H, –OH); ^{13}C-NMR (DMSO-d_6) δ 36.89, 54.89,120.22, 120.71, 123.50, 127.09, 128.87, 129.06, 129.16, 129.73, 133.10, 133.26, 138.63, 139.65, 140.32,159.84, 169.34, 173.32. MS (ES) *m/z* 395 $[MH]^+$; MW: 394 (calcd. for $C_{21}H_{18}N_2O_4S$).

*7.1.9.18. 3-Phenyl-2(*R,S*)-{2-[(pyridine-2-carbonyl)-amino]-benzoylamino}-propionic acid (**18**).* Purification method: trituration with hot AcOEt; TLC (AcOEt/MeOH 2:1)—Rf: 0.35; m.p. 224–225 °C; ^{1}H-NMR (DMSO-d_6) δ 3.22 (m, 2H, –CH_2–CH<); 4.73 (m, 1H, –CH<); 7.05–8.97 (m, 13H, Ar); 9.01 (d, 1H, –NH–CH<); 12.62 (s, 1H, –NH–); 13.01 (b, 1H, –OH); ^{13}C-NMR (DMSO-d_6) δ 37.02, 54.64, 121.23, 122.27, 123.06, 123.71, 127.02, 127.74, 128.80, 129.07, 129.72, 132.71, 138.68, 138.78, 149.45, 150.29, 163.18, 168.51, 173.54. MS (ES) *m/z* 390 $[MH]^+$; MW: 389 (calcd. for $C_{22}H_{19}N_3O_4$).

*7.1.9.19. 3-Phenyl-2(*R,S*)-{2-[(pyridine-3-carbonyl)-amino]-benzoylamino}-propionic acid (**19**).* Purification method: trituration with hot AcOEt; TLC (AcOEt/MeOH 2:1)—Rf: 0.28; m.p. 219–220 °C; ^{1}H-NMR (DMSO-d_6) δ 3.23 (m, 2H, –CH_2–CH<); 4.73 (m, 1H, –CH<); 7.09–9.02 (m, 13H, Ar); 9.18 (d, 1H, –NH–CH<); 12.18 (s, 1H, –NH–); 13.00 (b, 1H, –OH); ^{13}C-NMR (DMSO-d_6) δ 36.95, 54.86, 120.95, 121.14, 123.95, 124.69, 127.06, 128.82, 129.05, 129.75, 130.71, 133.20, 135.42, 138.59, 139.45, 148.70, 153.29, 163.55, 169.26, 173.29. MS (ES) *m/z* 390 $[MH]^+$; MW: 389 (calcd. for $C_{22}H_{19}N_3O_4$).

*7.1.9.20. 3-Phenyl-2(*R,S*)-{2-[(pyridine-4-carbonyl)-amino]-benzoylamino}-propionic acid (**20**).* Purification method: trituration with hot AcOEt; TLC (AcOEt/MeOH

2:1)—Rf: 0.24; m.p. 251–252 °C; ^{1}H-NMR (DMSO-d_6) δ 3.24 (m, 2H, –CH_2–CH<); 4.73 (m, 1H, –CH<); 7.05–8.85 (m, 13H, Ar); 9.18 (d, 1H, –NH–CH<); 12.29 (s, 1H, –NH–); ^{13}C-NMR (DMSO-d_6) δ 37.02, 55.00, 121.02, 121.13, 121.42, 124.19, 127.04, 128.81, 129.06, 129.78, 133.23, 138.70 139.23, 142.03, 151.50, 163.39, 169.13, 173.32. MS (ES) *m/z* 390 $[MH]^+$; MW: 389 (calcd. for $C_{22}H_{19}N_3O_4$).

*7.1.9.21. 3-Phenyl-2(*R,S*)-{2-[(pyrazine-2-carbonyl)-amino]-benzoylamino}-propionic acid (**21**).* Purification method: trituration with hot AcOEt; TLC (AcOEt/MeOH 2:1)—Rf: 0.31; m.p. 228–229 °C; ^{1}H-NMR (DMSO-d_6) δ 3.23 (m, 2H, –CH_2–CH<); 4.74 (m, 1H, –CH<); 7.02–9.32 (m, 13H, Ar and –NH–CH<); 12.67 (s, 1H, –NH–); 12.99 (b, 1H, –OH); ^{13}C-NMR (DMSO-d_6) δ 36.98, 54.71, 212.22, 121.90, 124.10, 127.00, 128.79, 129.13, 129.74, 132.90, 138.62, 138.68, 144.26, 144.61, 145.11, 148.64, 162.08, 168.53, 173.43. MS (ES) *m/z* 391 $[MH]^+$; MW: 390 (calcd. for $C_{21}H_{18}N_4O_4$).

*7.1.9.22. 3-Phenyl-2-{2-[(4,5,6,7-tetrahydro-1*H*-indole-2-carbonyl)-amino]-benzoylamino}-propionic acid (**22**).* Trituration with hot abs. EtOH afforded the titled compound in 78% yield. TLC (AcOEt/MeOH 2:1)—Rf: 0.48; m.p. 265–266 °C dec.; ^{1}H-NMR (DMSO-d_6) δ 1.74 (m, 4H, –CH_2–); 2.54 (m, 4H, –CH_2–); 3.19 (m, 2H, –CH_2–CH<); 4.75 (m, 1H, –CH<); 6.39 (s, 1H, Ar); 7.10–7.40 (m, 6H, Ar); 7.55 (t, 1H, Ar); 7.78 (d, 1H, Ar); 8.64 (d, 1H, Ar); 9.10 (d, 1H, –NH–CH<); 11.37 (s, 1H, –NH–); 11.74 (s, 1H, –NH–); 12.95 (b, 1H, –OH); ^{13}C-NMR (DMSO-d_6) 22.33, 22.48, 22.61, 23.19, 36.03, 54.02, 108.94, 117.73, 118.32, 119.22, 121.32, 123.77, 126.31, 128.07, 128.18, 128.90, 132.32, 132.95, 137.90, 140.01, 158.55, 168.64, 172.62. MS (ES) *m/z* 432 $[MH]^+$; MW: 431 (calcd. for $C_{25}H_{25}N_3O_4$).

*7.1.9.23. 3-Phenyl-2-{2-[(1,4,5,6-tetrahydro-cyclopenta[b] pyrrole-2-carbonyl)-amino]-benzoylamino}-propionic acid (**23**).* Trituration with hot abs. EtOH afforded the titled compound in 86% yield. TLC (AcOEt/MeOH 2:1)—Rf: 0.47; m.p. 265 °C dec.; ^{1}H-NMR (DMSO-d_6) δ 2.33 (m, 2H, –CH_2–); 2.59 (m, 4H, –CH_2–); 3.22 (m, 2H, –CH_2–CH<); 4.69 (m, 1H, –CH<); 6.38 (s, 1H, Ar); 7.05–7.35 (m, 6H, Ar); 7.50 (t, 1H, Ar); 7.72 (d, 1H, Ar); 8.58 (d, 1H, Ar); 9.06 (d, 1H, –NH–CH<); 11.47 (s, 1H, –NH–); 11.71 (s, 1H, –NH–); 12.95 (b, 1H, –OH); ^{13}C-NMR (DMSO-d_6) δ 24.43, 24.66, 28.59, 35.97, 54.04, 105.44, 118.32, 119.19, 121.31, 126.31, 127.11, 128.07, 128.62, 128.89, 132.33, 137.88, 140.00, 142.53, 158.51, 168.68, 172.60. MS (ES) *m/z* 418 $[MH]^+$; MW: 417 (calcd. for $C_{24}H_{24}N_3O_4$).

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

The binding parameters for the substances under investigation (IC_{50} values and $P = 0.05$ fiducial limits) were determined by regression analysis of competition curves.

7.2.1. [^{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. [23]. Drug displacing experiments were carried out by incubating acinar cells, [^{125}I]BH-CCK-8 (25 pM final concentration) and competitors in 0.5 ml total volume at 37 °C for 30 min, in shaking bath. At the end of incubation 1 ml of ice-cold Hepes-Ringer buffer (10 mM Hepes, 118 mM NaCl, 1.13 mM $MgCl_2$, 1.28 $CaCl_2$, 1% BSA, 0.2 mg/ml soybean trypsin inhibitor, pH 7.4) was added and the tubes were centrifuged 5 min at 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.

7.2.2. [^{125}I]BH-CCK-8 receptor binding assay in guinea pig cerebral cortices

Membranes from guinea pig cerebral cortices, were prepared as previously described [23]. Protein concentration was determined according to Bradford [28], using bovine serum albumin (BSA) as standard.

The binding experiments were performed in assay buffer containing 10 mM Hepes, 118 mM NaCl, 4.7 mM KCl, 5.0 mM $MgCl_2$, 1.0 mM EGTA, pH 6.5 and supplemented with 0.2 mg/ml bacitracin. The incubation of membranes suspension with labeled ligand and inhibitors was carried out in a microtiter 96-wells filter plate (Multiscreen, Millipore Inc, Bedford, MA) with integral Whatman GF/B membrane filters. Aliquot of membranes (0.5 mg of protein/ml) were added to each well, containing [^{125}I]BH-CCK8 (25 pM), in a final volume of 250 μl. The non-specific binding of iodinated peptide was defined in the presence of 1 μM CCK-8, accounting of 20% of total binding. 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).

7.3. Molecular modeling

A first set of optimized conformations for all the compounds was obtained by a simple Monte Carlo search. All the

rotatable bonds, including the amide bonds, were allowed to rotate in order to generate the starting set of geometries. Each bond was twisted by 10° torsional increments randomly and the initial set was thus obtained. The geometries were optimized first using molecular mechanics calculations with the Amber forcefield [29]; the optimizations were carried out with the Polak–Ribiere conjugate gradient algorithm to a gradient of 0.001 kcal/Å mol. The first 10 conformations obtained at this first step were then submitted to a further refinement, and their geometries were reoptimized with a semiempirical calculation using the AM1 [30] Hamiltonian as implemented in Sybyl 6.8 [1]. The SCF convergence limit for the UHF calculation was set to full accuracy.

All the calculations were carried out on a Silicon Graphics O_2 workstation.

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