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Orginal article

# Design, synthesis, and biological evaluation of non-peptidic ligands at the Xenopus laevis skin-melanocortin receptor

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**Abstract**

Taking the tripeptide D-Trp-Arg-Leu-$NH_2$ as a lead for a Xenopus laevis skin-melanocortin (MC) receptor antagonist, thirteen non-peptidic compounds were synthesized and biologically evaluated at Xenopus laevis melanophores. Six competitive antagonists (shown by Schild analysis) and one partial agonist were identified with moderate activity ($IC_{50}$: 5–10 μM). Tryptophanamides with aliphatic side chains were inactive whereas basic residues restored activity. Introducing an imidazole residue yielded partial agonist activity (EC50: 32 μM). Interestingly, constraining the inactive *S*-tryptophan-isoamylamide to a β-carboline ring yielded an MC receptor antagonist (**42**). The specificity for MC receptors was tested at various G-protein coupled receptors. In conclusion, the synthesis of non-peptidic MC receptor antagonists is described which may serve as lead compounds for further studies.


*Keywords:* Melanocortin receptor; Non-peptidic ligands; Xenopus laevis melanophores; Partial agonist

## 1. Introduction

Melanocortin (MC) receptors belong to the large superfamily of seven transmembrane domains containing receptors, the G-protein coupled receptors [1]. A group of endogenous neuropeptides derived from the prohormone pro-opiomelanocortin, α-, β-, and γ-melanocyte stimulating hormone (MSH) and adrenocorticotropic hormone (ACTH), act as physiological ligands [1]. These neuropeptides possess the His-Phe-Arg-Trp-consensus sequence [2,3] and transmit a broad variety of physiological functions through five different subtypes (MC1-MC5 receptors) [1]. The MC1 receptor, expressed in melanocytes and leukocytes, is implicated in skin pigmentation and inflammation [1,4,5]. The MC2 receptor was recognized as the ACTH receptor stimulating glucocorticoid synthesis in the adrenal gland [6]. The MC3 and MC4 receptors are expressed in the brain and involved in the control of feeding behavior [1,7,8]. Finally, the MC5 receptor is expressed in a variety of peripheral tissues regulating exocrine gland function [9].

The use of physiological ligands like MSH for in vivo studies is limited due to enzymatic degradation and a lack of selectivity. Further, a detailed evaluation of MC receptor physiology has been hindered by a lack of selective MSH antagonists. Therefore, a number of peptide analogues partially retaining the α-MSH-sequence have been synthesized some of which display prolonged activity and enzymatic stability [10–12]. Some peptide analogues display subtype selectivity for MC3 or MC4 receptors [13] or for MC1/5 [3,13–15]. Peptide analogues are mainly agonists [3,13,16]. However, Kavarana et al. have described selective and potent cyclic peptide antagonists at human MC3 and MC4 receptors [13]. At the MC1 receptor, non-peptide agonists have been identified [3], and di- or tripeptides antagonising the amphibian MC receptor [17]. To our

*Abbreviations:* cAMP, cyclic 3′,5′-adenosinemonophosphate; CDI, carbonyldiimidazole; GPCR, G-protein coupled receptor; MC, melanocortin; MSH, melanocyte stimulating hormone; RT, room temperature; THF, tetrahydrofuran.

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knowledge, a potent and selective non-peptidic MC1 antagonist with low molecular mass and metabolic stability has not yet been described.

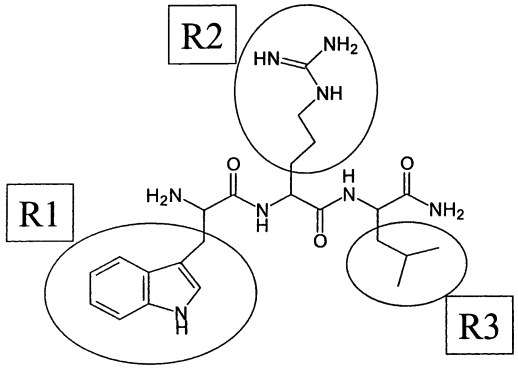
Quillan et al. described the combinatorial synthesis and evaluation of a series of tripeptides at the Xenopus laevis skin-MC receptor [18]. $IC_{50}$ values of some tripeptides are listed in Fig. 1, [18]. D-Trp-Arg-Leu-$NH_2$ was the most potent compound with an $IC_{50}$ of 0.62 μM (Fig. 1). Replacing D-Trp by D-Phe reduced the antagonistic effect by a factor of 7. Switching from D-Trp to L-Trp in position 1, reduced the $IC_{50}$ 161-fold. Changing the amino acid in position 2 from a basic (L-Arg) to a neutral residue (L-Leu) also resulted in a massive loss of activity (Fig. 1). Most interestingly, reducing the size of the aliphatic side chain of the third amino acid by a methylene group (L-Leu → L-Val) or by deleting the isopropyl-residue (L-Leu → L-Ala) resulted in a massive or complete loss of activity (Fig. 1).

The purpose of our study was to test whether compounds derived from the tripeptide D-Trp-Arg-Leu, and based on a non-peptidic structure (deletion of one amino acid and of amino or carboxy functions, or condensation to a β-carboline ring system) would still retain inhibitory activity at MC receptors. We therefore synthesized a series of thirteen non-peptidic compounds and the two dipeptides D(R)-Trp-Leu-$OCH_3$ (**16**) and D(R)-Trp-Leu-$NH_2$ (**17**) as controls and evaluated their biological activity at the Xenopus laevis skin-MC receptor.

## 2. Chemistry

Three classes of compounds were synthesized: indolyl-3-propionamides (Fig. 2), tryptophanamides (Fig. 3A, B, C, D), and a β-carbolinecarboxamide (Fig. 4). The carboxy-moiety of indolyl-3-propionic acid (**1**) was activated by carbonyldiimidazole (CDI) according to Staab et al. (Fig. 2) [19]. Reaction with isoamylamine (**2**), *S*-Leu-$OCH_3$ (**3**), or *S*-Leu-$NH_2$ (**4**) resulted in the indolyl-3-propionamides **5**–**7**, and subsequent cleavage of the ester moiety in **6** yielded the carboxylated derivative **8** (Fig. 2). Synthesis of the tryptophanamides started with Boc-protection of *R*- or *S*-Trp (**9**) using di-tertiary-butyl-dicarbonate according to Moroder et al. [20], activation of **11** by CDI (Fig. 3A) [19], and subsequent acylation of various amines at room temperature (RT) (Fig. 3A, B, C, D). Amines to be acylated were isoamylamine (**2**) (Fig. 3A), *S*-Leu-$OCH_3$ (**3**) or *S*-Leu-$NH_2$ (**4**) (Fig. 3B), and mono-Boc-protected alkyldiamines (**21**–**23**). Mono-Boc-protection of alkyldiamines was performed according to Krapcho starting from the respective alkyldiamines (**18**–**20**) (Fig. 3C) [21]. Further, alkylamines containing an additional ternary nitrogen or imidazole residue (**30**–**32**) were used (Fig. 3D). In the final step, the Boc-protection group was cleaved by standard procedures [22] resulting in the desired compounds (**R**)-**13**, (**S**)-**13**, the dipeptides D-Trp-Leu-$OCH_3$ and D-Trp-Leu-$NH_2$ (**16**, **17**), **27**–**29**, and **36**–**38** (Fig. 3A, B, C, D). Fig. 4 depicts the synthesis of (*S*)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid-(3-methylbutyl)-amide (**42**). The β-carboline3-carboxylic acid (**39**) was obtained according to Coutts et al. starting with *S*-Trp ((**S**)-**9**) (Fig. 4) [23]. After Boc-protection of the basic nitrogen [20] and activation of the carboxy function with CDI [19], isoamylamine was acylated, and cleavage of the Boc-protection group [22] yielded the target compound **42**.

If possible, compounds were prepared as hydrochlorides (**27**–**29**, **36**–**38**). However, if compounds were too hygroscopic, the free bases were isolated ((**R**)-**13**, (**S**)-**13**, **16**, **17**, **42**). Compound (**S**)-**13** and **17** are already described in the literature but experimental data were not given [18,22,24]. We therefore provide analytical data for these compounds in the Section 6. Compound **38** was already synthesized by Supuran and Scozzafava as the *S*-enantiomer, and analytical data were presented [25]. For our study, we synthesized the *R*-enantiomer. Our compound was isolated as dihydrochloride. Data from the elemental analysis are in accordance with literature data [25]. $^1$H-NMR-shifts given by us are slightly different from literature because we measured




| R1 | R2 | R3 | $IC_{50}$, μM |
|---|---|---|---|
| D-Trp | L-Arg | L-Leu | 0.62 ± 0.15 |
| D-Trp | L-Arg | **L-Nle** | 0.93 ± 0.22 |
| **D-Phe** | L-Arg | L-Nle | 4.4 ± 1.2 |
| D-Trp | **L-Leu** | L-Nle | 48 ± 2.2 |
| **L-Trp** | L-Arg | L-Nle | 100 ± 2 |
| D-Trp | L-Arg | **L-Val** | 237 ± 100 |
| D-Trp | L-Arg | **L-Ala** | > 1000 |

Fig. 1. Tripeptides as Xenopus laevis skin-MC receptor antagonists. Data are from Quillan et al. [18].

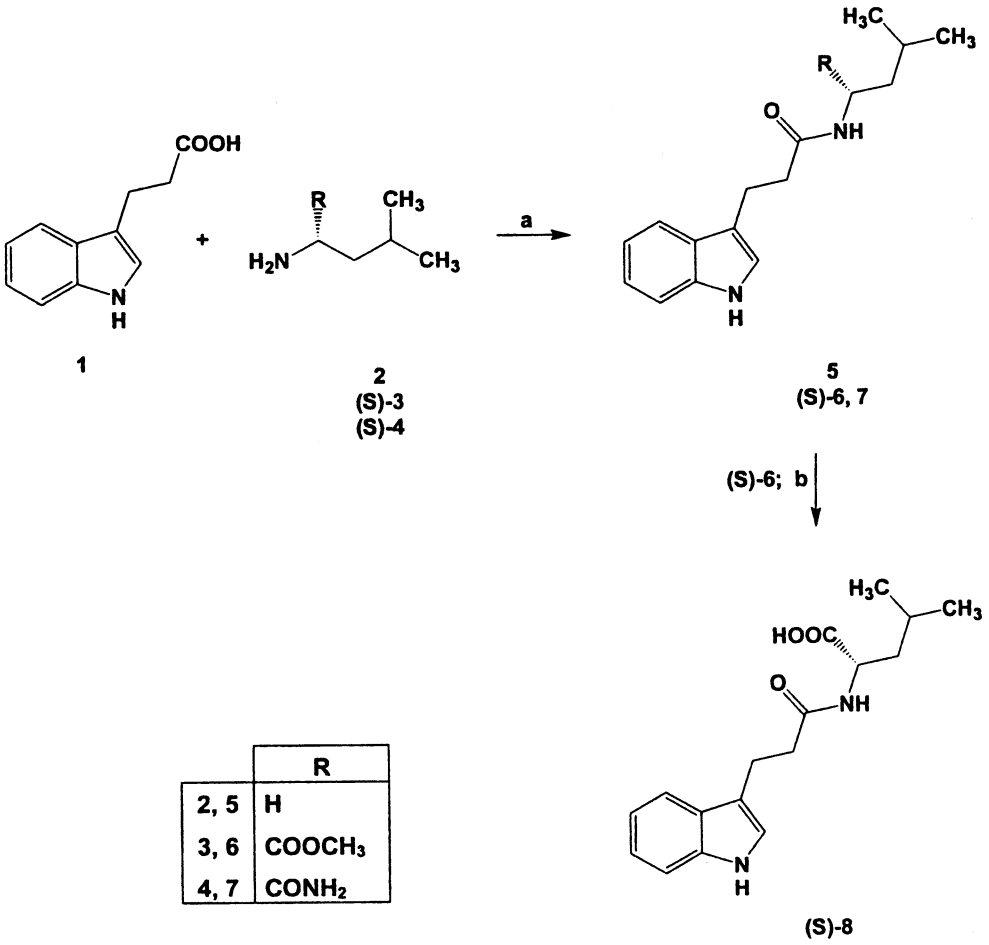



Fig. 2. Synthesis of indolyl-3-propionamides. (a) CDI, THF, RT; (b) NaOH, RT.

the free base of **38** whereas Supuran and Scozzafava measured in trifluoracetic acid [25]. Further, optical rotation was measured but could not be compared since literature did not reveal the optical rotation of the *S*-enantiomer. Compounds **27** and **28** were of insufficient purity to obtain elemental analysis results within the expected range of $\pm 0.4\%$ of the theoretical value. However, since **27** and **28** were only of modest potency at Xenopus laevis MC receptors ($IC_{50}$ values of 8.6 and $\sim 10$ μM, respectively, Table 1), **27** and **28** were not further purified. Instead, MALDI-MS analysis performed as recently described confirmed the molecular mass of the two compounds [26]. Additional signals in the MALDI-MS spectra were in accordance with the masses of mono-Boc-protected derivatives of **27** and **28**. We therefore conclude that Boc-deprotection was not completed under the chosen reaction conditions. The degree of purity of **27** and **28** was estimated as ca. 90% by semi-quantitative thin layer chromatography (TLC). Elemental analyses of all other target compounds (**5**–**8**, (**R**)-**13**, (**S**)-**13**, **16**, **17**, **29**, **36**-**38**, **42**) were within the expected range ($\pm 0.4\%$ of the theoretical value). NMR results of all target compounds were consistent with the assigned structures.

## 3. Pharmacology

The purpose of this study was to search for non-peptidic skin-MC receptor antagonists to allow a therapeutic evaluation of this MC receptor, which is involved in skin pigmentation, fur coloration, and possibly development of melanoma cancer [27]. A simple, robust, and easily accessible biological test system for skin-MC receptor activity is the Xenopus laevis melanophore assay [17,18]. The assay is based on the ability of Xenopus laevis melanophores to show an easily measurable response to changes in cyclic 3′,5′-adenosinemonophosphate (cAMP) accumulation. At low intracellular cAMP concentrations, melanin is stored in granula (aggregated state). Thus, melanophores show high transmission for visible (VIS) light (Fig. 5). Upon stimulation of the $G_s$-coupled MC receptor in melanophores of Xenopus laevis, the cAMP concentration increases and leads to a dispersion of melanin. Thus, cells appear dark and display low transmission for VIS light which can easily be measured with an Elisa Reader (Fig. 5). Prior to stimulation with α-MSH, Xenopus laevis melanophores were pretreated with 10 nM melatonin to start an assay in the

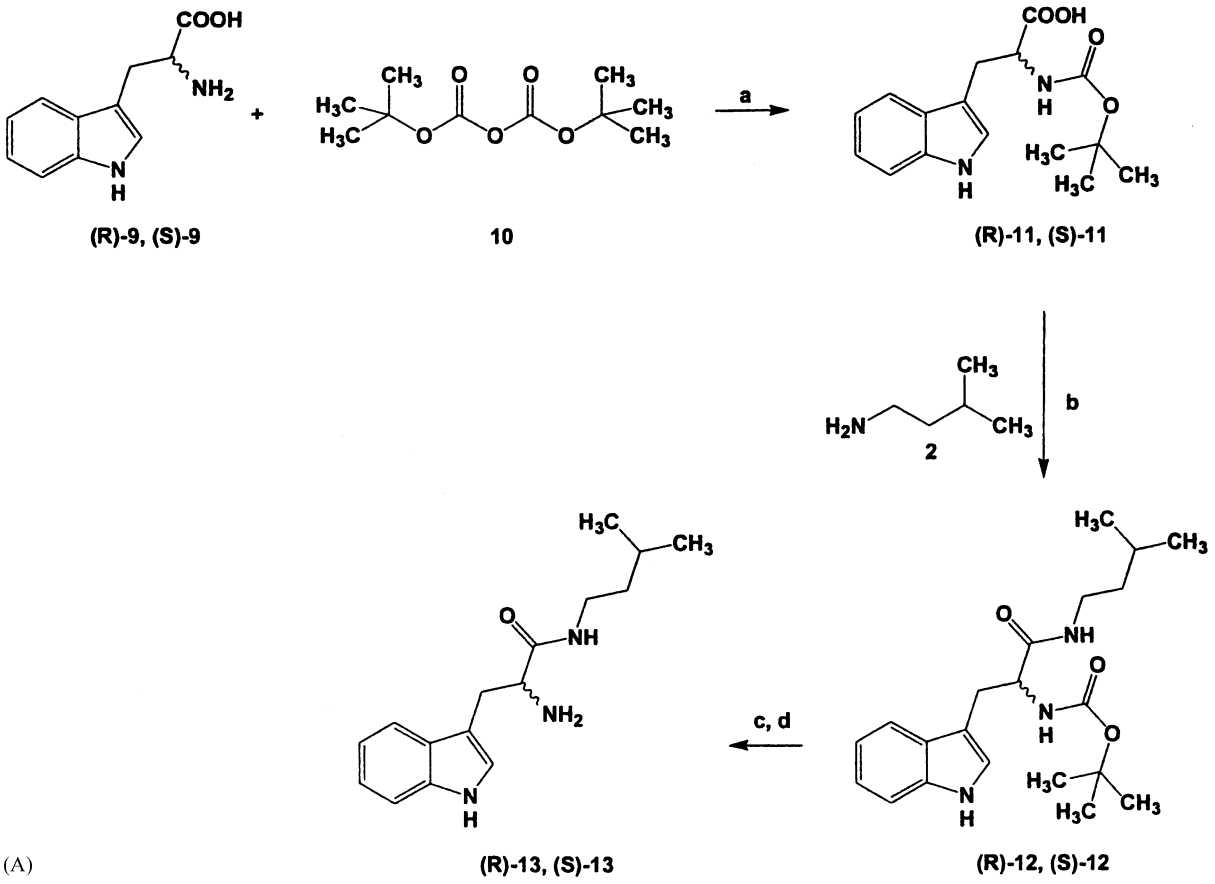
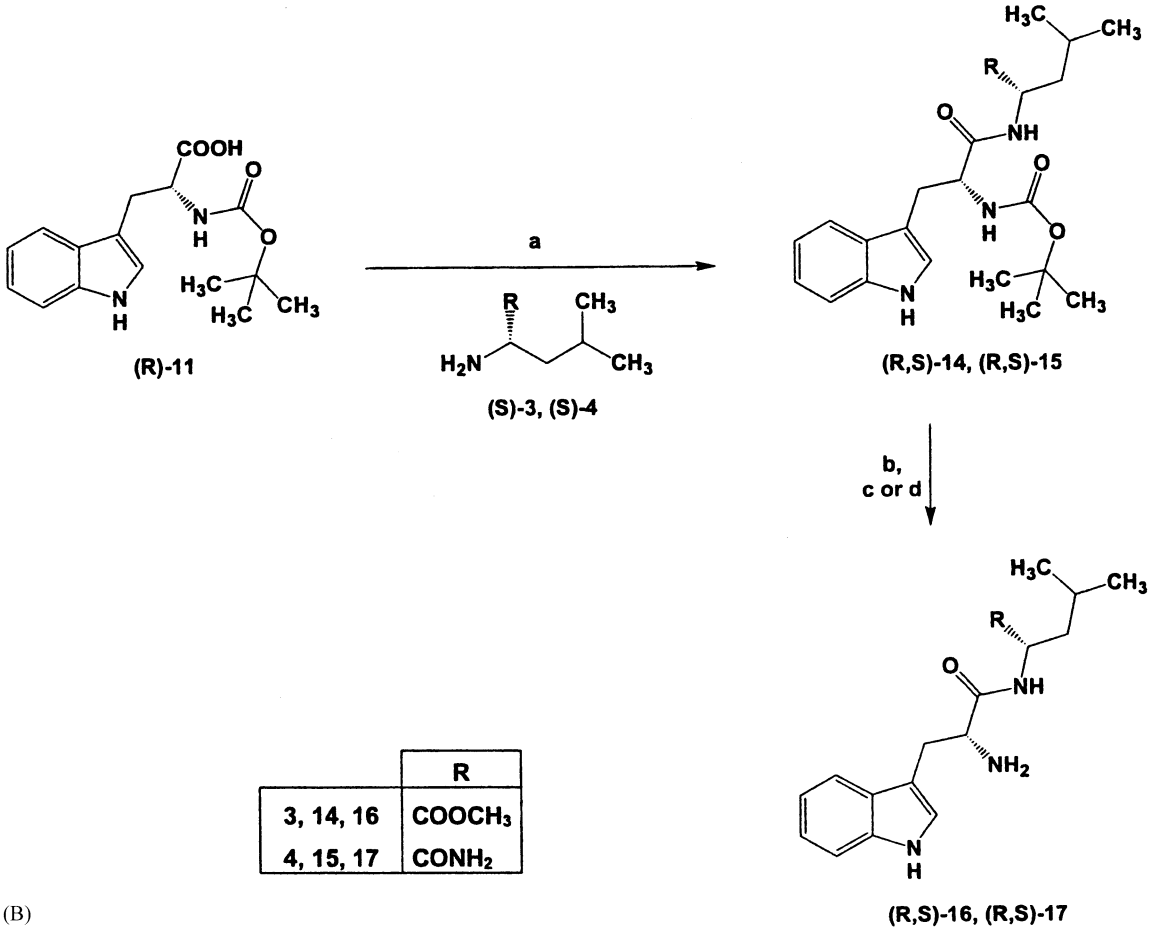



Fig. 3. Synthesis of tryptophanamides. (A) Isoamylamine as aminocompound. (a) THF/water/NaOH 10%, 0 °C; (b) CDI, THF, RT; (c) 2 N HCl/acetic acid; (d) NaOH; (B) (*S*)-Leucine-derivatives as aminocompounds. (a) CDI, THF, RT; (b) 2 N HCl/ethyl acetate; (c) NaOH; (d) diethylether; (C) Diamines as aminocompounds. (a) THF, 0 °C; (b) CDI, THF, RT; (c) 2 N HCl/diethylether; (D) Amines with ternary nitrogen as aminocompounds. (a) CDI, THF, RT; (b) 2 N HCl/ethylacetate.

aggregated state (low absorbance). Treatment of melanophores in the aggregated state with α-MSH resulted in a concentration-dependent pigment dispersion and thus increase in VIS-absorbance (Fig. 6). The $EC_{50}$ of α-MSH was estimated as $0.21 \pm 0.01$ nM ($n = 4$). The Xenopus laevis melanophore assay system can therefore be used to screen for agonistic and antagonistic behavior of a test compound at skin-MC receptors. Starting from the aggregated state after pretreatment with 10 nM melatonin, agonism of a test compound at the MC receptor can be detected by an increase in absorbance. Indeed, one of our test compounds, **38**, displayed agonistic activity as shown in the kinetic experiment in Fig. 7A at a concentration of 100 μM. Other compounds were inactive when compared to control (buffer) (Fig. 7A). Starting again from the aggregated state of melanophores after pretreatment with 10 nM melatonin, compounds were tested at 100 μM for their ability to antagonise the effect of 0.6 nM α-MSH ($\sim 3 \times$ $EC_{50}$) (Fig. 7B). α-MSH alone (= control) reached maximum absorption within the first 10 min. As an example, **27** and **42** completely antagonised the α-MSH response whereas **5** was inactive, and **38** only slightly antagonised the α-MSH effect (Fig. 7B).

## 4. Results

A series of thirteen non-peptidic compounds and two dipeptides derived from the tripeptide D-Trp-Arg-Leu-$NH_2$ were synthesized (Figs. 2–4). Three of the compounds have already been described in the literature, among them the dipeptide D-Trp-Leu-$NH_2$ **17** [18]. However, none of the compounds except the dipeptide D-Trp-Leu-$NH_2$ **17** has been evaluated at MC receptors. Here, quantitative data for the fifteen synthesized compounds on their activity at the Xenopus laevis skin-MC receptor are presented in Table 1. None of the indolyl-3-propionamides (**5**–**8**) was active up to 100 μM (Table 1). *R*- or *S*-tryptophan-isoamylamide ((**R**)-**13**, (**S**)-**13**) and the dipeptides D-Trp-Leu-$NH_2$ (**17**) and D-Trp-Leu-$OCH_3$ (**16**) were also inactive at 100 μM. However, introducing a basic residue into the aliphatic side chain regained activity of the compounds. **27**–**29**,




| | R |
|---|---|
| 3, 14, 16 | $COOCH_3$ |
| 4, 15, 17 | $CONH_2$ |

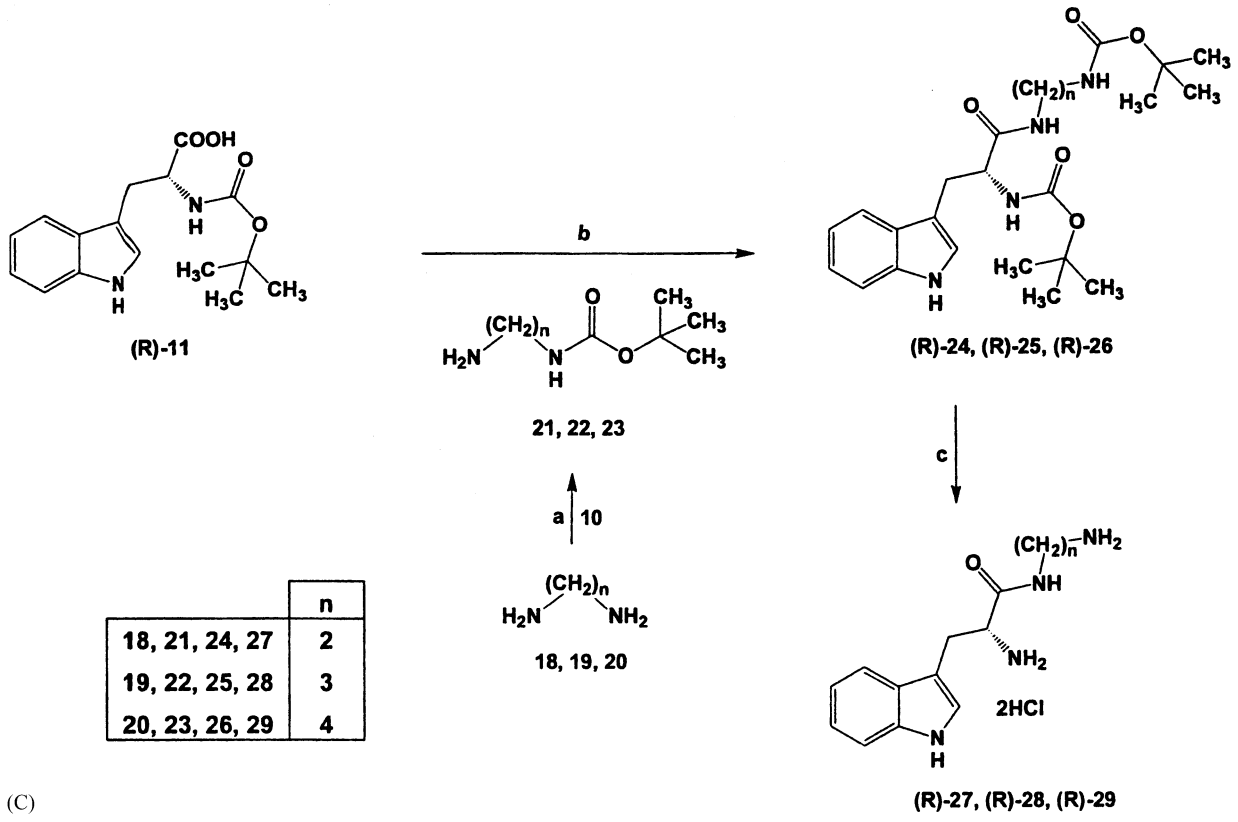
(B)

Fig. 3 (*Continued*)

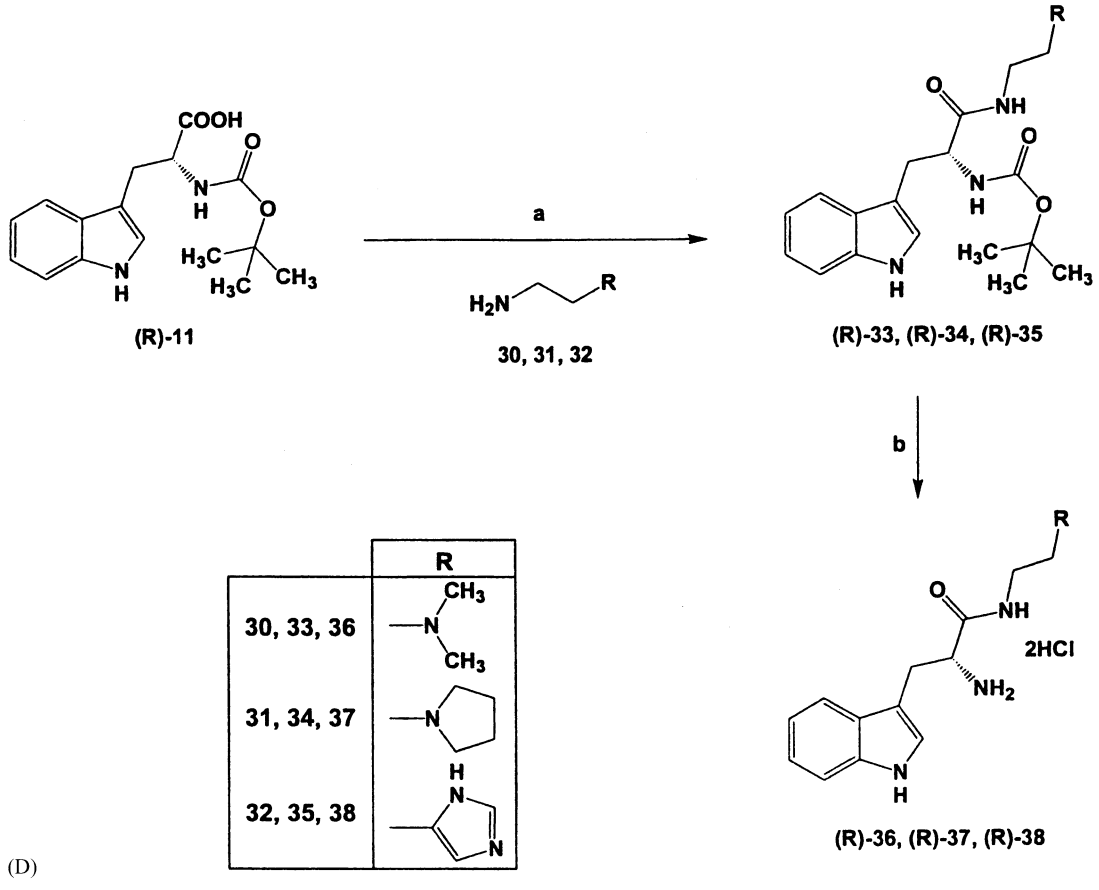
**36**, and **37** antagonised the α-MSH effect with an $IC_{50}$ between 5 and 10 μM (Table 1). Interestingly, introducing constraints into the flexible but inactive compound (**S**)-**13** by forming a β-carboline ring system resulted in the active compound **42**. The non-peptidic compound **42** was—together with **27** and **37**—among the most potent compounds (Table 1). These three compounds were tested for competitive antagonism by performing a Schild plot analysis. Fig. 8A shows as an example the rightward shift of the concentration-response curve of α-MSH in the presence of increasing concentrations of **42**. The corresponding Schild analysis is displayed in Fig. 8B. The $pA_2$ value was calculated as $5.47 \pm 0.06$ corresponding to a $K_d$ value of 3.4 μM. The slope was not significantly different from unity. $pA_2$ values for compounds **27** and **37** are listed in Table 1.

Compound **38** combining the structures of *R*-Trp and histamine emerged as partial agonist at the Xenopus laevis MC receptor. This compound has been described earlier, but it was tested for activation of carbonic anhydrase and not mentioned in context with MC receptors [25]. A first hint for partial agonist behavior of **38** appeared in Fig. 7A and B. **38** mimicked the effect of α-MSH (Fig. 7A) but could not completely block the α-MSH response at 100 μM (Fig. 7B). Concentration-response curves of **38** for agonism and antagonism revealed its partial agonist behavior. The $EC_{50}$ was 32 μM. The efficacy of **38** was lower than that of α-MSH: **38** reached only 86% of the maximum α-MSH response. The $IC_{50}$ of **38** (blockade of α-MSH response) was estimated as about 10 μM (Table 1).

Active compounds **27**, **37**, **38** and **42** were tested for their selectivity for the MC receptor. None of the compounds interfered with β-adrenoceptors, dopamine-, μ-opioid-, muscarinic acetylcholine M1-, arginine vasotocin-, or melatonin-receptors (data not shown). When melanophores were not pretreated with melatonin (i.e. starting from a [light induced] dispersed state), α-MSH and **38** only slightly increased a further dispersion of the melanophores (agonistic effect at MC receptors), all other compounds were inactive (data not shown). Thus, it was shown that none of the test compounds




(C)




(D)

Fig. 3 (*Continued*)

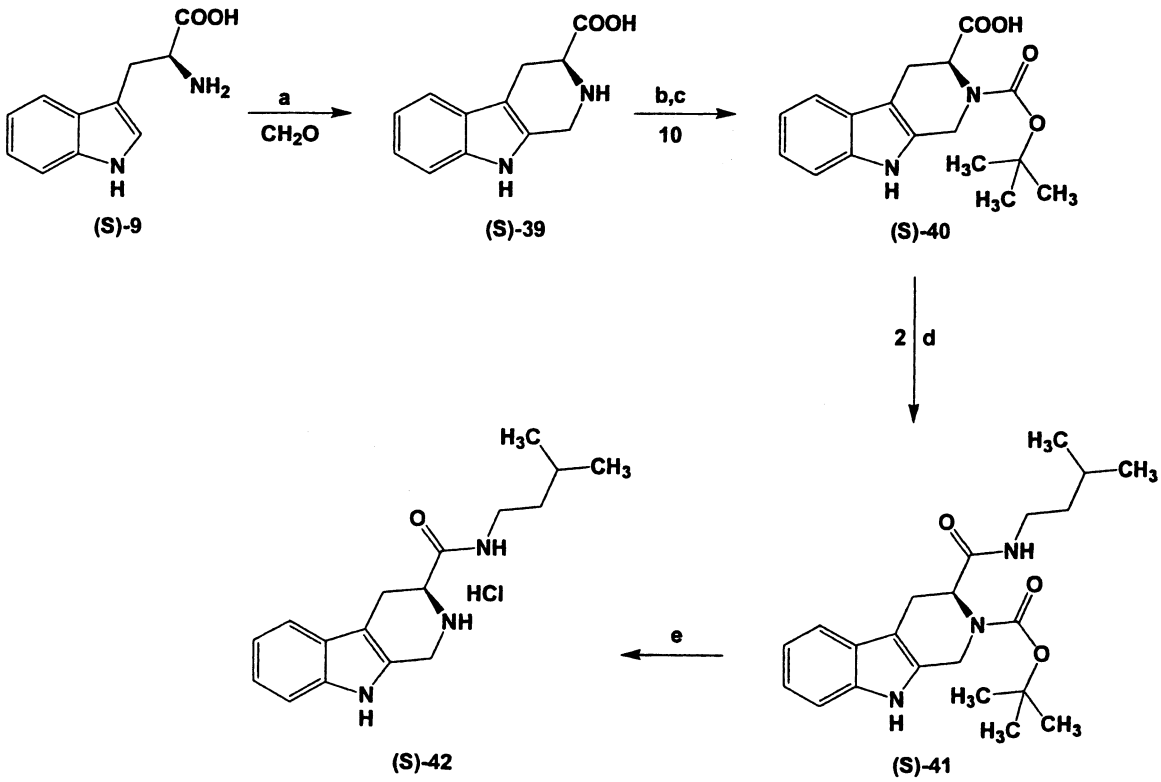



Fig. 4. Synthesis of (*S*)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid-(3-methylbutyl)-amide. (a) $H^+$; (b) THF, 0 °C, 60 min; (c) THF, RT, 18 h; (d) CDI, THF, RT; (e) 2 N HCl/ethylacetate.

stimulates a $G_i$ coupled receptor expressed in melanophores which would have led to an increase in aggregation.

Table 1
Potencies of the synthesized compounds at Xenopus laevis skin-MC receptors

| Compound | $IC_{50}$ ± S.D., (μM) | p$A_2$ |
|---|---|---|
| **5** | > 100 | nd |
| **6** | > 100 | nd |
| **7** | > 100 | nd |
| **8** | > 100 | nd |
| **(R)-13** | > 100 | nd |
| **(S)-13** | > 100 | nd |
| **16** | > 100 | nd |
| **17** | > 100 | nd |
| **27** | 8.6 ± 3.0 | 5.10 ± 0.15 |
| **28** | ~ 10 | nd |
| **29** | ~ 10 | nd |
| **36** | ~ 10 | nd |
| **37** | 5.2 ± 1.6 | 5.43 ± 0.09 |
| **38** [a] | ~ 10 | nd |
| **42** | 7.3 ± 2.2 | 5.47 ± 0.06 |

nd, not determined. For compounds with an $IC_{50}$ of > 100 μM or ~ 10 μM, one experiment with 4 replicates was performed. More potent compounds ($IC_{50}$ of < 10 μM) were evaluated in 3 independent experiments each with 3–4 replicates. Reversible competitive antagonism was shown by Schild analysis. p$A_2$ values from 3 independent experiments are given (slopes were not significantly different from unity).

[a] Partial agonist.

## 5. Discussion

To our knowledge, this is the first report on non-peptidic skin-MC receptor antagonists. Of a series of fifteen compounds, six compounds were competitive antagonists, and one compound turned out as partial agonist at the Xenopus laevis MC receptor (Table 1). The potency of the compounds was rather moderate ($IC_{50}$ between 5 and 10 μM). The biological test system (Xenopus laevis melanophores) was already applied by Quillan et al. for evaluation of combinatorial di- and tripeptides [18] and a structure-based search for MC receptor ligands [17]. $EC_{50}$ values for α-MSH in this study and previously obtained were of similar magnitude (0.21 or 0.75 nM, respectively) [17].


Fig. 5. Two different states of Xenopus laevis melanophores. Low cAMP concentrations (i.e. stimulation of $G_i$ coupled receptors) lead to an aggregated state. High cAMP concentrations (i.e. stimulation of $G_s$ coupled receptors) lead to a dispersed state. Shift from one state to the other is fully reversible.

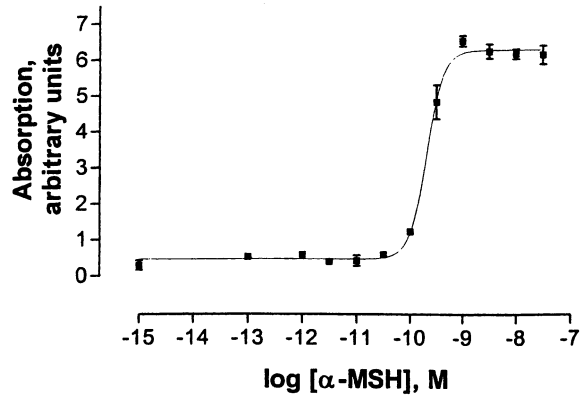



Fig. 6. Concentration-response curve of α-MSH at Xenopus laevis melanophores expressing MC receptors after pre-treatment of cells with 10 nM melatonin.

Indolyl-3-propionamides not containing a basic residue (**5**–**8**), *R*- or *S*-tryptophan-isoamylamide ((**R**)-**13**, (**S**)-**13**), and D(R)-Trp-Leu-$OCH_3$ and D(R)-Trp-Leu-$NH_2$ (**16**, **17**) were all inactive at Xenopus MC receptors at 100 μM (Table 1). However, introducing basic residues into the *N*-alkyl side chain of the tryptophanamides resulted in compounds displaying a moderate blockade of MC receptors (**27**–**29**, **36**, **37**). Variations of the methylene spacer length for a primary amino group (2-4 methylene groups) did not change the activity of the compounds (**27**–**29**) (Table 1). Switching from a primary (**27**) to a tertiary dimethylamino group (**36**) did not change the potency. However, replacing the dimethylamino by a pyrrolidine residue (**37**) increased the potency by twofold (Table 1).

Introducing a β-carboline ring system into the inactive compound (**S**)-**13** (*S*-tryptophan-isoamylamide) resulted in the constrained and active compound **42** (Fig. 4, Table 1). The constrained compound **42** may serve as a lead compound for computer-assisted modeling. Interestingly, among the series of basic substituted *R*-tryptophanamides, a switch from antagonistic to partial agonistic behavior occurred when introducing a histamine moiety resulting in the known compound **38** (Fig. 7A, B). Testing a limited selectivity profile showed no interaction of the active compounds with β-adrenoceptors, dopamine-, μ-opioid-, muscarinic acetylcholine M1-, arginine vasotocin-, melatonin-, or other $G_i$ coupled receptors expressed in melanophores, and thus a specificity of the active compounds for antagonism (or partial agonism in case of **38**) at MC receptors.

Xenopus laevis melanophores are a simple test system for potential MC receptor ligands [18]. However, affinities of compounds at the amphibian MC receptor and at human skin-MC receptors, i.e., MC1 receptors, do not always correlate [17]. CTAP and the anticancer peptide both blocked the amphibian MC receptor whereas only CTAP but not the anticancer peptide blocked human MC1-receptors [17]. The tripeptide D-Trp-Arg-Leu-$NH_2$, the starting point for this study, blocked amphibian as well as human MC1-receptors [18]. However, the four active compounds in this study (**27**, **37**, **38** and **42**) need to be evaluated at human MC receptors.

In conclusion, we present here the first non-peptidic amphibian MC receptor antagonists. Starting from the tripeptide D-Trp-Arg-Leu-$NH_2$ (Fig. 1), the structure was further condensed (deletion of one amino acid), and the peptidic character was abolished (deletion of the carboxy group in the second amino acid, and introducing a basic side chain or a β-carboline ring). The potency of the resulting non-peptidic compounds dropped ~10-fold compared to the tripeptide D-Trp-Arg-Leu-$NH_2$. However, with the β-carboline ring containing compound **42**, we have introduced a novel, small, and constrained structure as a lead compound for designing MC receptor antagonists. The synthesis and evaluation of further related compounds is under investigation.

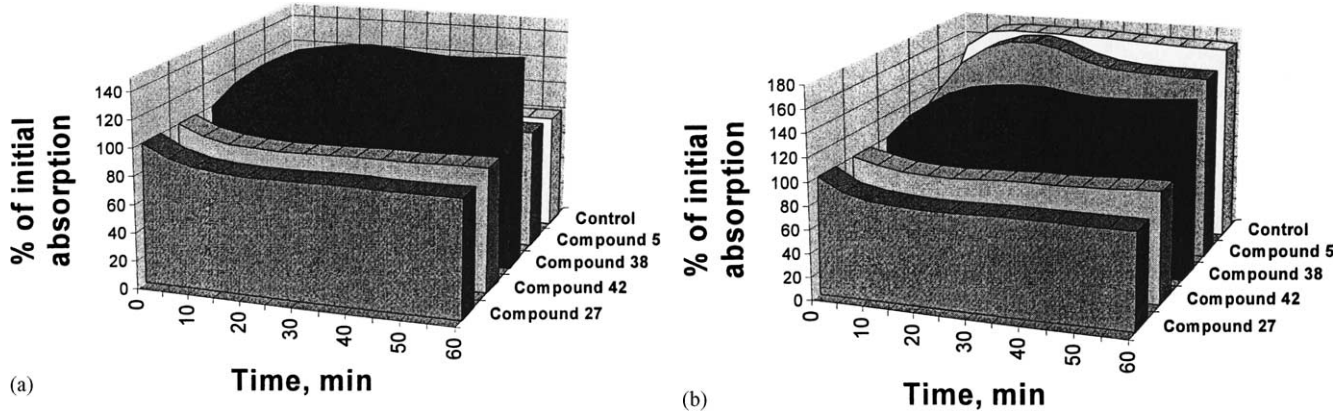



Fig. 7. (A) Time course of absorption after addition of test compounds (100 μM) to Xenopus laevis melanophores pretreated with 10 nM melatonin. Control is addition of buffer only. (B) Time course of absorption after simultaneous addition of 0.6 nM α-MSH and test compounds (100 μM) to Xenopus laevis melanophores pretreated with 10 nM melatonin. Control is addition of α-MSH only.

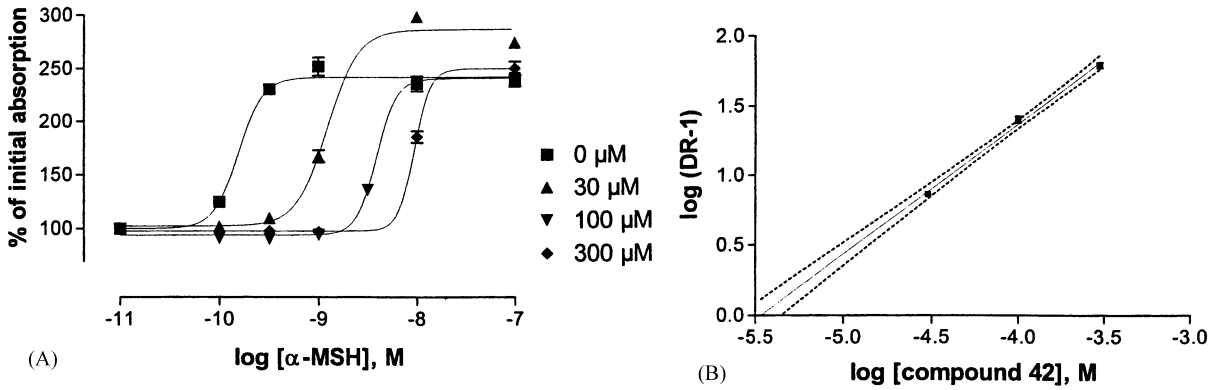



Fig. 8. (A) Effect of increasing concentrations of compound **42** on the position of the concentration-response curve of α-MSH at Xenopus laevis MC receptors. Data shown are representative for a typical experiment out of 3 each with 3–4 replicates. (B) Schild plot analysis of compound **42** at Xenopus laevis MC receptors. α-MSH was used as agonist. Dashed lines show 95% confidence intervals. Data points presented are average from 3 experiments each performed with 3 replicates. $EC_{50}$ values of α-MSH were as follows ($n = 3$–4): $EC_{50}$ (absence of **42**): 0.21 ± 0.01 nM; $EC_{50}$ (30 μM **42**): 1.24 ± 0.14 nM; $EC_{50}$ (100 μM **42**): 3.92 ± 0.33 nM; $EC_{50}$ (300 μM **42**): 9.43 ± 2.50 nM.

## 6. Experimental protocols

### 6.1. Chemistry

Melting points (m.p.) were determined in open capillaries on a Gallenkamp m.p. apparatus and are uncorrected. IR spectra were recorded with a FT-IR-spectrophotometer 'Paragon 1000' from Perkin Elmer. $^1$H-NMR spectra were recorded on a Bruker AC200 (200 MHz) and a Bruker WM500 (500 MHz) spectrometer in DMSO-$d_6$. Chemical shifts are given in $\delta$ (ppm) using tetramethylsilane as internal standard. TLC was performed on 5 × 10 cm$^2$ plates precoated with silica gel GF254 (E. Merck, Darmstadt, Germany). Elution solvent mixture were: toluene:acetone:ammonia 65:35:5 (system 1); methylenechloride:ethanol:acetic acid 90:10:1 (system 2); butanol:water:acetic acid 40:10:10 (system 3); toluene:acetone:ammonia 20:75:5 (system 4); methylenechloride:acetone:acetic acid 50:50:1 (system 5); methylenechloride:acetone:acetic acid 80:20:1 (system 6). Detection was performed with short wavelength UV light and iodine for visualisation. Optical rotation was measured with a Polarimeter P 1000 (A. Krüss Optronic GmbH) and corrected to 25 °C. Elemental analysis (C, H, N) was performed on a Vario EL apparatus from Elementar. The analytical results of the elements were within ±0.4% of the theoretical values, unless otherwise indicated. MALDI-MS was performed as described in [26]. Matrix used was 2,5-dihydroxybenzoic acid.

### 6.2. Synthesis of indolyl-3-propionamides **5**–**8**

3-Indolepropionic acid (**1**), isoamylamine (**2**) and 1,1′-carbonyldiimidazole (CDI) were obtained from Acros, and (*S*)-leucinemethylester-HCl (**3**) and (*S*)-leucinamide-HCl (**4**) were obtained from Fluka.

#### 6.2.1. 3-(1H-Indol-3-yl)-N-(3-methylbutyl)propionamide (**5**)

3-Indolepropionic acid (**1**) (1.9 g, 10 mmol) was dissolved in 50 mL tetrahydrofuran (THF), and CDI (1.6 g, 10 mmol) was added. After stirring at RT for 3 h, isoamylamine (**2**) (0.9 g, 10 mmol) was added to the solution and stirred overnight. The solvent was evaporated under reduced pressure, and the residue was dissolved in 100 mL ethyl acetate, washed three times with 20 mL sulfuric acid (1 N), three times with 20 mL aqueous sodium hydroxide (10%) and three times with 20 mL brine, dried ($MgSO_4$) and evaporated to a solid (**5**) Yield: 0.8 g (29%); m.p. 77 °C; TLC: $R_f$ 0.71 (system 2); $[a]_D^{25\,°C} = +3.11°$ ($c = 0.6$; methanol); IR $\nu_{max}$ (KBr, cm$^{-1}$) 1639; $^1$H-NMR: $\delta$ 0.83 (6H, ds, $CH_3$), 1.25 (2H, mc, $(CH_3)_2$–CH–C$H_2$–), 1.51 (1H, mc, $(CH_3)_2$–*CH*–), 2.40 (2H, mc, indole–*CH*$_2$–), 2.90 (2H, mc, –CO–*CH*$_2$–), 3.05 (2H, mc, –NH–*CH*$_2$–), 6.95 (1H, ddd, $J = 7.7$, 7.0, 0.9, indole–H5), 7.04 (1H, ddd, $J = 7.7$, 7.0, 0.9, indole–H6), 7.06 (1H, s, indole–H2), 7.31 (1H, d, $J = 7.7$, 0.9, indole–H7), 7.50 (1H, d, $J = 7.7$, 0.9, indole–H4), 7.71 (1H, t, $J = 6.6$, –CO–*NH*–), 10.71 (1H, s, indole–NH); Anal. ($C_{16}H_{22}N_2O$) C, H, N.

#### 6.2.2. (S)-N$^2$-[3-(1H-Indole-3-yl-propanoyl)]leucinemethylester (**6**)

**6** was synthesized as described under Section 6.2.1 using *S*-leucinemethylester (**3**), which was released from *S*-leucinemethylester-hydrochloride with an equimolar amount of triethylamine. Yellow oil was obtained. Yield: 2.4 g (75%); TLC: $R_f$ 0.54 (system 5); $[a]_D^{25\,°C} = -46.77$ ($c = 0.64$; methanol); IR $\nu_{max}$ (KBr, cm$^{-1}$) 1654; $^1$H-NMR: $\delta$ 0.80 (3H, d, $J = 6.5$, –$CH_3$), 0.86 (3H, d, $J = 6.5$, –$CH_3$), 1.50 (3H, m, $(CH_3)_2$–*CH*– and $(CH_3)_2$–CH–*CH*$_2$–), 2.49 (2H, mc, indole–*CH*$_2$), 2.90 (2H, mc, –CO–*CH*$_2$–), 3.61 (3H, s, –COO*CH*$_3$), 4.30 (1H, mc, –NH–*CH*–), 6.96 (1H, ddd, $J = 7.6$, 7.6, 1.0, indole–H5), 7.05 (1H, ddd, $J = 7.6$, 7.6, 1.0, indole–

H6), 7.07 (1H, d, indole–H2), 7.31 (1H, dd, $J = 7.6, 1.0$, indole–H7), 7.51 (1H, dd, $J = 7.6$, indole–H4), 8.19 (1H, d, $J = 7.6$, –CO–*NH*), 10.73 (1H, s, indole–NH); Anal. ($C_{18}H_{24}N_2O_3$) C, H, N.

### 6.2.3. (*S*)-$N^2$-[3-(1*H*-Indole-3-yl-propanoyl)]leucineamide (**7**)

**7** was synthesized as described under Section 6.2.1 using *S*-leucineamide (**4**), which was released from *S*-leucineamide-hydrochloride with an equimolar amount of triethylamine. The obtained residue was recrystallized from methylenechloride. Yield: 2.0 g (64%); m.p. 132 °C; TLC: $R_f$ 0.45 (system 2); $[a]_D^{25\,°C} = -41.89$ ($c = 0.71$; methanol); IR $\nu_{max}$ (KBr, $cm^{-1}$) 1662; $^1$H-NMR: $\delta$ 0.80 (3H, d, $J = 6.2$, $-CH_3$), 0.84 (3H, d, $J = 6.2$, $-CH_3$), 1.41 (2H, mc, $(CH_3)_2$–CH–$CH_2$–), 1.49 (1H, mc, $(CH_3)_2$–*CH*–), 2.49 (2H, mc, indole–$CH_2$), 2.90 (2H, mc, –CO–$CH_2$–), 4.24 (1H, mc, –NH–*CH*–), 6.87 and 7.24 (2x1H, 2xs, –CO–$NH_2$), 6.95 (1H, ddd, $J = 7.5$, 7.5, 1.0 indole–H5), 7.04 (1H, ddd, $J = 7.5$, 7.5, 1.1, indole–H6), 7.07 (1H, d, $J = 2.3$, indole–H2), 7.31 (1H, dd, $J = 7.5$, 1.0, indole–H7), 7.52 (1H, dd, $J = 7.5$, 1.1, indole–H4), 7.85 (1H, d, $J = 8.0$, –CO–*NH*–), 10.71 (1H, s, indole–NH); Anal. ($C_{17}H_{23}N_3O_2$) C, H, N.

### 6.2.4. (*S*)-$N^2$-[3-(1*H*-Indole-3-yl-propanoyl])leucine (**8**)

**6** was dissolved in 100 mL THF/acetone (1:1). After cooling with ice, 30 mL aqueous sodium hydroxide (1 N) were added and the stirred solution warmed up to RT. After stirring another 60 min at RT, 150 mL ice-cooled water were added and the solution was acidified with hydrochloric acid (36%) until a pH of 1 was reached. An orange oil precipitated at the wall of the flask, and was dissolved in 30 mL aqueous sodium hydroxide (10%) and the solution was washed four times with methylenechloride. Then, the solution was acidified with hydrochloric acid (36%) and **8** was extracted from the aqueous phase with methylenechloride. The organic phase was dried ($MgSO_4$). The solvent was removed under reduced pressure and a fawn solid was obtained. Yield: 2.1 g (45%); m.p. 133 °C; TLC: $R_f$ 0.51 (system 6); $[a]_D^{25\,°C} = -47.00$ ($c = 0.59$; methanol); IR $\nu_{max}$ (KBr, $cm^{-1}$) 1715; $^1$H-NMR: $\delta$ 0.80 (3H, d, $J = 6.5$, $-CH_3$), 0.86 (3H, d, $J = 6.5$, $-CH_3$), 1.48 (2H, mc, $(CH_3)_2$–CH–$CH_2$–), 1.55 (1H, mc, $(CH_3)_2$–*CH*–), 2.49 (2H, mc, indole–$CH_2$), 2.90 (2H, mc, –CO–$CH_2$–), 4.30 (1H, mc, –NH–*CH*–), 6.95 (1H, ddd, $J = 7.3$, 8.1, 1.0 indole–H5), 7.04 (1H, ddd, $J = 7.3$, 8.1, 1.0, indole–H6), 7.08 (1H, d, $J = 2.2$, indole–H2), 7.31 (1H, d, $J = 8.1$, indole–H7), 7.51 (1H, d, $J = 7.3$, indole–H4), 8.06 (1H, d, $J = 7.9$, –CO–*NH*–), 10.71 (1H, s, indole–NH), 12,41 (1H, s, –COO*H*); Anal. ($C_{17}H_{22}N_2O_3$) C, H, N.

### 6.3. Synthesis of tryptophanamides (**R**)-**13**, (**S**)-**13**, **16**, **17**, **27**–**29**, **36**–**38**

(*R*)-tryptophan ((**R**)-**9**), di-tertiary-butyl-dicarbonate (**10**), isoamylamine (**2**), ethylendiamine (**18**), 1,3-diaminopropane (**19**), *N*-(2-aminoethyl)-pyrrolidin (**31**), histamine-di-HCl (**32**) and 1,1′-carbonyldiimidazole (CDI) were obtained from Acros. *S*-leucinemethylester-HCl (**3**), *S*-leucineamide-HCl (**4**), 1,4-diaminobutane (**20**) and 2-dimethylaminoethylamine (**30**) were obtained from Fluka, and (*S*)-tryptophan ((**S**)-**9**) was obtained from Aldrich.

(**R**)-**11** and (**S**)-**11** were synthesized as described in Moroder et al. method A using (**R**)-**9** and (**S**)-**9** as amino acids [20]. The synthesis of (**R**)-**12** and (**S**)-**12** was performed as described under Section 6.2.1 using (**R**)-**11** and (**S**)-**11** instead of **1**. **14** and **15** were synthesized as described under Section 6.2.1 using **3** and **4** as amino compounds and (**R**)-**11** as carboxylic acid compound. **21**–**23** were synthesized according to Krapcho and co-workers using **18**–**20** as starting material [21]. **24**–**26** were synthesized as described under Section 6.2.1 using **21**–**23** as amino compounds and (**R**)-**11** as carboxylic acid compound. 33–35 were synthesized as described under Section 6.2.1 using **30**–**32** as amino compounds and (**R**)-**11** as carboxylic acid compound.

### 6.3.1. *N*-(3-methylbutyl)-(*S*)-tryptophanamide ((**S**)-**13**)

5 mL of 2 N HCl in acetic acid were added to $N^1$-(tertiary-butoxycarbonyl)-$N^2$-(3-methylbutyl)-*S*-tryptophanamide ((**S**)-**12**) (1.0 g, 2,7 mmol). After 20 min, 10 mL water and 50 mL sodium hydroxide 10% were added. The supernatant was sucked off from the precipitate and dried under vacuum. Recrystallisation from methylenechloride gave a white solid ((**S**)-**13**). Yield: 0.3 g (34%); m.p. 111 °C; TLC: $R_f$ 0.22 (system 1); $[a]_D^{25\,°C} = +24.42$ ($c = 0.68$; methanol); IR $\nu_{max}$ (KBr, $cm^{-1}$) 1734; $^1$H-NMR: $\delta$ 0.83 (6H, ds, $CH_3$), 1.22 (2H, q, $J = 7.2$, $(CH_3)_2$–CH–$CH_2$), 1.48 (1H, m, $(CH_3)_2$–*CH*–), 2.73 (1H, dd, $J_{AB} = 14.3$, $J_{AX} = 8.0$, indole–$CH_{2(A)}$–), 3.02 (1H, dd, $J_{AB} = 14.3$, $J_{BX} = 4.4$, indole–$CH_{2(B)}$–), 3.06 (2H, m, –NH–$CH_2$–), 3.40 (1H, dd, $J = 8.0$, 4.4, –CO–$CH_X$–), 6.95 (1H, ddd, $J = 7.7$, 7.5, 1.0, indole–H5), 7.04 (1H, ddd, $J = 7.7$, 7.5, 1.0, indole–H6), 7.12 (1H, d, $J = 2.2$, indole–H2), 7.31 (1H, d, $J = 7.7$, indole–H7), 7.54 (1H, d, $J = 7.7$, indole–H4), 7.71 (1H, t, $J = 4.40$, –CO–*NH*), 10.79 (1H, s, indole–NH); Anal. ($C_{16}H_{22}N_2O$) C, H, N.

### 6.3.2. *N*-(3-methylbutyl)-(*R*)-tryptophanamide ((**R**)-**13**)

(**R**)-**13** was synthesized as described under Section 6.3.1 using (**R**)-**12** instead of (**S**)-**12**. Yield: 0.2 g (29%); m.p. 111 °C; TLC: $R_f$ 0.24 (system 1); $[a]_D^{25\,°C} = -38.55°$ ($c = 0.59$; methanol); IR $\nu_{max}$ (KBr, $cm^{-1}$)

1646; $^1$H-NMR: $\delta$ 0.83 (6H, ds, $CH_3$), 1.22 (2H, q, $J$ = 7.2, $(CH_3)_2$–CH–$CH_2$), 1.47 (1H, m, $(CH_3)_2$–$CH$–), 2.73 (1H, dd, $J_{AB}$ = 14.4, $J_{AX}$ = 8.0, indole–$CH_{2(A)}$–), 3.03 (1H, dd, $J_{AB}$ = 14.4, $J_{BX}$ = 5.0, indole–$CH_{2(B)}$–), 3.05 (2H, m, –NH–$CH_2$–), 3.39 (1H, dd, $J$ = 8.0, 5.0, –CO–$CH_X$–), 6.95 (1H, ddd, $J$ = 7.6, 7.3, 0.9, indole–H5), 7.04 (1H, ddd, $J$ = 7.6, 7.3, 0.9, indole–H6), 7.12 (1H, d, $J$ = 2.2, indole–H2), 7.31 (1H, d, $J$ = 7.6, indole–H7), 7.54 (1H, d, $J$ = 7.6, indole–H4), 7.70 (1H, t, $J$ = 5.5, –CO–$NH$), 10.79 (1H, s, indole–NH); Anal. ($C_{16}H_{22}N_2O$) C, H, N.

### 6.3.3. (*R,S*)-$N^2$-[2-amino-3-(1*H*-indole-3-yl-propanoyl)]leucinemethylester (**16**)

5 mL of 2 N HCl in acetic acid were added to (*R*,*S*)-$N^2$-[2-tertiary butoxycarbonylamino-3-(1*H*-indole-3-yl-propanoyl)]leucinemethylester (**14**) (2.0 g, 4,7 mmol). After 20 min 100 mL diethylether were added. The precipitate was sucked off and dried under vacuum. Yield:1.1 g (65%); m.p. 115 °C; TLC: $R_f$ 0.78 (system 3); $[a]_D^{25\,°C} = -38.46°$ ($c$ = 0.78; methanol); IR $\nu_{max}$ (KBr, $cm^{-1}$) 1684; $^1$H-NMR: $\delta$ 0.72 (3H, d, $J$ = 6.62, $CH_3$), 0.80 (3H, d, $J$ = 6.62, $CH_3$), 1.32 (1H, m, $(CH_3)_2$–$CH$–), 1.41 (2H, m, $(CH_3)_2$–CH–$CH_2$), 3.17 (1H, dd, $J_{AB}$ = 14.4, $J_{AX}$ = 7.4, indole–$CH_{2(A)}$–), 3.24 (1H, dd, $J_{AB}$ = 14.4, $J_{BX}$ = 6.8, indole–$CH_{2(B)}$–), 3.64 (3H, s, –COO$CH_3$), 4.07 (1H, m, indole–$CH_2$–$CH_X$–), 4.24 (1H, m, $COOCH_3$–$CH$–), 7.01 (1H, dd, $J$ = 7.5, 7.4, indole–H5), 7.10 (1H, dd, $J$ = 7.5, 7.4, indole–H6), 7.24 (1H, d, $J$ = 2.2, indole–H2), 7.37 (1H, d, $J$ = 7.5, indole–H7), 7.67 (1H, d, $J$ = 7.4, indole–H4), 8.95 (1H, d, $J$ = 7.6, –CO–$NH$), 11.07 (1H, s, indole–NH); Anal. ($C_{18}H_{25}N_3O_3$) C, H, N.

### 6.3.4. (*R,S*)-$N^2$-[2-amino-3-(1*H*-indole-3-yl-propanoyl)]leucineamide (**17**)

5 mL of 2 N HCl in ethyl acetate were added to (*R*,*S*)-$N^2$-[2-tertiary butoxycarbonylamino-3-(1*H*-indole-3-yl-propanoyl)]leucineamide (**15**) (0.4 g, 1.0 mmol). After 20 min, 10 mL water and 50 mL sodium hydroxide 10% were added. The precipitate was sucked off and dried under vacuum. Yield: 0.2 g (42%); m.p. 146 °C; TLC: $R_f$ 0.63 (system 3); $[a]_D^{25\,°C} = -61.22°$ ($c$ = 0.98; methanol); IR $\nu_{max}$ (KBr, $cm^{-1}$) 1669; $^1$H-NMR: $\delta$ 0.65 (3H, d, $J$ = 6.3, $CH_3$), 0.74 (3H, d, $J$ = 6.3, $CH_3$), 1.14 (1H, m, $(CH_3)_2$–$CH$–), 1.28 and 1.39 (2x1H, 2xm, $(CH_3)_2$–CH–$CH_2$), 3.13 (1H, dd, $J_{AB}$ = 14.2, $J_{AX}$ = 7.3, indole–$CH_{2(A)}$–), 3.22 (1H, dd, $J_{AB}$ = 14.2, $J_{BX}$ = 7.6, indole–$CH_{2(B)}$–), 4.11 (2H, m, indole–$CH_2$–$CH_X$– and –CO–NH–$CH$–), 7.00 (1H, ddd, $J$ = 7.6, 7.7, 0.9, indole–H5), 7.03 and 7.48 (2x1H, 2xs, –CO–$NH_2$), 7.09 (1H, ddd, $J$ = 7.6, 7.7, 0.9, indole–H6), 7.22 (1H, d, $J$ = 2.2, indole–H2), 7.31 (1H, d, $J$ = 7.7, indole–H7), 7.48 (1H, s, –CO–$NH_2$), 7.69 (1H, d, $J$ = 7.6, indole–H4), 8.73 (1H, d, $J$ = 8.5, –CO–$NH$), 11.06 (1H, s, indole–NH); Anal. ($C_{17}H_{24}N_4O_2$) C, H, N.

### 6.3.5. (*R*)-2-Amino-*N*-(2-aminoethyl)-3-(1*H*-indole-3-yl)-propionamide-dihydrochloride (**27**)

40 mL of 2 N HCl in ethyl acetate were added during 30 min drop-wise and under ice-cooling to (*R*)-[1-(2-tertiary-butoxycarbonylamino-ethylcarbamoyl)-2-(1*H*-indole-3-yl)-ethyl]-carbamic acid-tertiary-butylester (**24**) (0.4 g, 1.0 mmol). Then, 200 mL diethylether were added and the precipitate was sucked off immediately and dried under vacuum. Yield: 0.2 g (76%); m.p. 137 °C; TLC: $R_f$ 0.30 (system 4); $[a]_D^{25\,°C} = -18.18°$ ($c$ = 0.55; methanol); IR $\nu_{max}$ (KBr, $cm^{-1}$) 1684; $^1$H-NMR (free base): $\delta$ 2.55 (2H, m, –CO–NH–$CH_2$–$CH_2$–$NH_2$), 2.74 (1H, dd, $J_{AB}$ = 14.1, $J_{AX}$ = 8.0, indole–$CH_{2(A)}$–), 3.05 (1H, dd, $J_{AB}$ = 14.1, $J_{BX}$ = 5.1, indole–$CH_{2(B)}$–), 3.13 (2H, m, –CO–NH–$CH_2$), 3.43 (1H, m, CO–$CH_X$–), 6.95 (1H, ddd, $J$ = 7.5, 7.5, 1.0, indole–H5), 7.04 (1H, ddd, $J$ = 7.5, 7.5, 1.0, indole–H6), 7.07 (1H, d, $J$ = 2.2, indole–H2), 7.31 (1H, d, $J$ = 7.5, indole–H7), 7.52 (1H, d, $J$ = 7.5, indole–H4), 7.89 (1H, t, $J$ = 7.3, –CO–$NH$), 10.82 (1H, s, indole–NH); MALDI-MS ($C_{13}H_{20}N_4OCl_2$ minus 2 HCl plus $H^+$): Calc. 247.156, Found 247.169; Anal. ($C_{13}H_{20}N_4OCl_2$) Calc. C 48.91, H 6.31, N 17.55, Found C 45.02, H 6.59, N 14.17%.

### 6.3.6. (*R*)-2-Amino-*N*-(3-aminopropyl)-3-(1*H*-indole-3-yl)-propionamide-dihydrochloride (**28**)

**28** was synthesized as described under Section 6.3.5 using (*R*)-[1-(3-tertiary-butoxycarbonylamino-propyl-carbamoyl)-2-(1*H*-indole-3-yl)-ethyl]-carbamic acid-tertiary-butylester (**25**). Yield: 0.2 g (48%); m.p. 149 °C; TLC: $R_f$ 0.22 (system 4); $[a]_D^{25\,°C} = -25.97°$ ($c$ = 0.77; methanol); IR $\nu_{max}$ (KBr, $cm^{-1}$) 1684; $^1$H-NMR: $\delta$ 1.69 (2H, m, –CO–NH–$CH_2$–$CH_2$–$CH_2$–$NH_2$), 2.71 (2H, m, –CO–NH–$CH_2$–$CH_2$–$CH_2$–$NH_2$), 3.15 (4H, m, –CO–NH–$CH_2$ and indole–$CH_2$–), 3.91 (1H, m, CO–$CH$–), 6.98 (1H, dd, $J$ = 7.5, 7.5, indole–H5), 7.07 (1H, dd, $J$ = 7.5, 7.5, indole–H6), 7.20 (1H, d, $J$ = 2.2, indole–H2), 7.32 (1H, d, $J$ = 7.5, indole–H7), 7.66 (1H, d, $J$ = 7.5, indole–H4), 8.13 (6H, s, $-NH_3$), 8.79 (1H, t, $J$ = 5.2, –CO–$NH$), 11.06 (1H, s, indole–NH); MALDI-MS: ($C_{14}H_{22}N_4OCl_2$ minus 2 HCl plus $H^+$): Calc. 261.172, Found 261.195; Anal. ($C_{14}H_{22}N_4OCl_2$) Calc. C 50.46, H 6.65, N 16.81, Found C 47.60, H 7.13, N 14.23%.

### 6.3.7. (*R*)-2-Amino-*N*-(4-aminobutyl)-3-(1*H*-indole-3-yl)-propionamide-dihydrochloride (**29**)

**29** was synthesized as described under Section 6.3.5 using (*R*)-[1-(4-tertiary-butoxycarbonylamino-butylcarbamoyl)-2-(1*H*-indole-3-yl)-ethyl]-carbamic acid-tertiary-butylester (**26**). Yield: 0.2 g (85%); m.p. 87 °C; TLC: $R_f$ 0.25 (system 4); $[a]_D^{25\,°C} = -11.24°$ ($c$ = 0.89; methanol); IR $\nu_{max}$ (KBr, $cm^{-1}$) 1675; $^1$H-NMR (free base): $\delta$ 1.32 (4H, m, –CO–NH–$CH_2$–$CH_2$–$CH_2$–$CH_2$–$NH_2$), 2.52 (2H, m, –CO–NH–$CH_2$–$CH_2$–$CH_2$–$CH_2$–$NH_2$), 2.73 (1H, dd, $J$ = 8.0, 14.3,

indole–$CH_{(B)}$–), 3.01 (3H, m, –CO–NH–$CH_2$ and indole–$CH_{(A)}$–), 3.40 (1H, m, CO–$CH$–), 6.98 (1H, ddd, $J = 7.6, 7.6, 0.9$, indole–H5), 7.04 (1H, ddd, $J = 7.6, 7.6, 0.9$, indole–H6), 7.20 (1H, s, indole–H2), 7.31 (1H, d, $J = 7.6$, indole–H7), 7.54 (1H, d, $J = 7.6$, indole–H4), 7.78 (1H, t, $J = 5.5$, –CO–$NH$), 10.80 (1H, s, indole–NH); Anal. ($C_{15}H_{24}N_4OCl_2$) C, H, N.

### 6.3.8. (*R*)-2-Amino-N-(2-dimethylaminoethyl)-3-(1H-indole-3-yl)-propionamide-dihydrochloride (**36**)

**36** was synthesized as described under Section 6.3.5 using (*R*)-[1-(2-dimethylaminoethylcarbamoyl)-2-(1*H*-indole-3-yl)-ethyl]-carbamic acid-tertiary-butylester (**33**). Yield: 0.3 g (80%); m.p. 101 °C; TLC: $R_f$ 0.36 (system 4); $[a]_D^{25\,°C} = -34.01°$ ($c = 0.59$; methanol); IR $\nu_{max}$ (KBr, $cm^{-1}$) 1683; $^1$H-NMR (free base): $\delta$ 2.08 (6H, m, $CH_3$), 2.19 (2H, m, $(CH_3)_2$–$CH_2$–), 2.75 (1H, dd, $J = 8.2, 14.2$, indole–$CH_{(B)}$–), 3.05 (1H, dd, $J = 4.7, 14.2$, indole–$CH_{(A)}$–), 3.12 (2H, m, –CO–NH–$CH_X$–), 3.42 (1H, m, CO–$CH$–), 6.95 (1H, dd, $J = 7.6, 7.6$, indole–H5), 7.04 (1H, dd, $J = 7.6, 7.6$, indole–H6), 7.15 (1H, d, $J = 2.2$, indole–H2), 7.31 (1H, d, $J = 7.6$, indole–H7), 7.53 (1H, d, $J = 7.6$, indole–H4), 7.75 (1H, t, $J = 5.4$, –CO–$NH$), 10.80 (1H, s, indole–NH); Anal. ($C_{15}H_{24}N_4OCl_2$) C, H, N.

### 6.3.9. (*R*)-2-Amino-3-(1H-indole-3-yl)-N-(2-pyrrolidin-1-yl-ethyl)-propionamide-dihydrochloride (**37**)

**37** was synthesized as described under Section 6.3.5 using (*R*)-[1-(2-(1*H*-indole-3-yl)-1-(2-pyrrolidin-1-yl ethylcarbamoyl)-ethyl]-carbamic acid tertiary-butylester (**34**). Yield: 0.3 g (94%); m.p. 134 °C; TLC: $R_f$ 0.38 (system 4); $[a]_D^{25\,°C} = -15.92°$ ($c = 0.63$; methanol); IR $\nu_{max}$ (KBr, $cm^{-1}$) 1683; $^1$H-NMR (free base): $\delta$ 1.64 (4H, m, pyrrolidin-H3 and -H4), 2.38 (6H, m, N-$CH_2$–), 2.75 (1H, dd, $J = 8.2, 14.1$, indole–$CH_{(B)}$–), 3.05 (1H, dd, $J = 4.9, 14.1$, indole–$CH_{(A)}$–), 3.14 (2H, m, –CO–NH–$CH_2$–), 3.41 (1H, m, CO–$CH_X$–), 6.95 (1H, ddd, $J = 7.7, 7.7, 0.9$, indole–H5), 7.04 (1H, ddd, $J = 7.7, 7.7, 0.9$, indole–H6), 7.14 (1H, d, $J = 2.2$, indole–H2), 7.32 (1H, d, $J = 7.7$, indole–H7), 7.53 (1H, d, $J = 7.7$, indole–H4), 7.78 (1H, t, $J = 5.4$, –CO–$NH$), 10.81 (1H, s, indole–NH); Anal. ($C_{17}H_{26}N_4OCl_2$) C, H, N.

### 6.3.10. (*R*)-2-Amino-N-[(2-(1H-imidazole-4-yl)-ethyl]-3-(1H-indole-3-yl)-)-propionamide-dihydrochloride (**38**)

**38** was synthesized as described under Section 6.3.5 using (*R*)-[1-(2-(1*H*-imidazole-4-yl)-ethylcarbamoyl)-2-(1*H*-indole-3-yl)-ethyl]-carbamic acid tertiary-butylester (**35**). Yield: 0.3 g (80%); m.p. 108 °C; TLC: $R_f$ 0.23 (system 4); $[a]_D^{25\,°C} = -31.70°$ ($c = 0.63$; methanol); IR $\nu_{max}$ (KBr, $cm^{-1}$) 1684; $^1$H-NMR (free base): $\delta$ 2.58 (2H, m, NH–$CH_2$–$CH_2$-imidazole), 2.73 (1H, dd, $J = 8.2, 14.2$, indole–$CH_{(B)}$–), 3.05 (1H, dd, $J = 4.4, 14.2$, indole–$CH_{(A)}$–), 3.28 (2H, m, –CO–NH–$CH_2$–), 3.41 (1H, m, CO–$CH_X$–), 6.72 (1H, s, imidazole-H5), 6.95 (1H, ddd, $J = 7.6, 7.6, 1.0$, indole–H5), 7.04 (1H, ddd, $J = 7.6, 7.6, 1.0$, indole–H6), 7.13 (1H, d, $J = 2.2$, indole–H2), 7.32 (1H, d, $J = 7.6$, indole–H7), 7.50 (1H, s, imidazole-H2), 7.54 (1H, d, $J = 7.6$, indole–H4), 7.92 (1H, t, $J = 5.7$, –CO–$NH$), 10.81 (1H, s, indole–NH); Anal. ($C_{16}H_{21}N_5OCl_2$) C, H, N.

## 6.4. Synthesis of (*S*)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid-(3-methylbutyl)-amide (**42**)

Di-tertiary-butyl-dicarbonate (**10**), isoamylamine (**2**), and CDI were obtained from Acros, and (*S*)-tryptophan ((**S**)-**9**) was obtained from Aldrich. **39** was synthesized as described by Coutts et al. [23]. **40** was synthesized as described by Moroder et al. [20]. **41** was synthesized as described in chapter Section 6.2.1 using **40** as the carboxylic acid compound. **42** was synthesized as described in chapter Section 6.3.3 using—instead of **14**—(*S*)-3-(3-methylbutylcarbamoyl)-1,3,4,9-tetrahydro-1*H*-β-carboline-2-carboxylic acid-tertiary-butylester (**41**) (1.0 g, 2.6 mmol). Yield: 0.6 g (77%); m.p. 150 °C; TLC: $R_f$ 0.67 (system 4); $[a]_D^{25\,°C} = -91.65°$ ($c = 0.98$; methanol); IR $\nu_{max}$ (KBr, $cm^{-1}$) 1653; $^1$H-NMR: $\delta$ 0.83 (6H, ds, $CH_3$), 1.33 (2H, m, $(CH_3)_2$–CH–$CH_2$), 1.58 (1H, m, $(CH_3)_2$–$CH$–), 2.63 (1H, dd, $J_{AB} = 15.1$, $J_{AX} = 9.8$, tetrahydro-β-carboline-$H_{4(A)}$), 2.85 (1H, dd, $J_{AB} = 15.1$, $J_{BX} = 4.5$, tetrahydro-β-carboline-$H_{4(B)}$), 3.13 (2H, m –CO–NH–$CH_2$–), 3.40 (1H, m, tetrahydro-β-carboline-H3), 3.91 (2H, m, tetrahydro-β-carboline-H1), 6.92 (1H, ddd, $J = 7.6, 7.6, 1.0$, tetrahydro-β-carboline-H6), 6.99 (1H, ddd, $J = 7.6, 7.6, 1.0$, tetrahydro-β-carboline-H7), 7.25 (1H, d, $J = 7.6$, tetrahydro-β-carboline-H8), 7.35 (1H, d, $J = 7.6$, tetrahydro-β-carboline-H5), 7.80 (1H, t, $J = 5.7$, –CO–$NH$), 10.64 (1H, s, tetrahydro-β-carboline-NH9); Anal. ($C_{17}H_{24}N_3OCl$) C, H, N.

## 6.5. Pharmacology

### 6.5.1. Cell culture

Xenopus laevis melanophore cells were prepared as previously described [17]. Melanophore cells were grown in culture medium consisting of 5 parts L-15 medium (Sigma), 2 parts fetal bovine serum (Sigma), 3 parts deionised-distilled water, 100 μg $mL^{-1}$ streptomycin, and 100 U $mL^{-1}$ penicillin G. Cells were incubated at RT under normal air condition.

### 6.5.2. Xenopus laevis melanophore MC receptor assay

MC receptor assays were performed in 96-well plates by measuring absorbance (630 nm) through a monolayer of Xenopus laevis melanophores using a BT2000 Microplate Reader (FisherBiotech, Pittsburgh, PA). When melanophores reached 100% confluency, culture medium was replaced by Krebs-HEPES buffer (118 mM

NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM $KH_2PO_4$, 4.2 mM $NaHCO_3$, 11.7 mM D-Glucose, 1.3 mM $CaCl_2$, 10 mM HEPES, pH 7.4, containing 0.5% bovine serum albumin). Preincubations in Krebs-HEPES buffer were performed in the presence or absence of 10 nM melatonin (Sigma) for 90 min to start assays from either an aggregated (low absorbance) or dispersed (high absorbance) state of the melanophores. Then, test compounds were added at a fixed concentration (100 μM) for screening purposes, or in increasing concentrations (for monitoring concentration-response curves) in the presence or absence of 0.6 nM α-MSH, and absorption was measured every 5 min for 60 min. The percent of initial absorption (% $A_i$) was calculated according to the formula: % $A_i = 100(A_t/A_i)$ where $A_t$ is the absorption at the timepoint $t$, and $A_i$ is the initial absorption ($t = 0$).

### 6.5.3. Data analysis

Concentration-response curves were constructed using nonlinear regression curve fit (sigmoidal dose response equation with variable hill slope) and was performed by using Prism software 3.0 from GaphPad. $IC_{50}$ is the inhibitory concentration 50% of an antagonist, $EC_{50}$ is the effective concentration 50% of an agonist (e.g. α-MSH). Schild-plot analysis was performed to demonstrate reversible competitive antagonism of the compounds at the amphibian MC receptor. If an antagonist obeys the Schild equation, the resulting graph is a straight line with a slope not significantly different from unity (Eq. (1)) [28]:

$$\log [DR - 1] = \log [\text{antagonist}] + pA_2 \quad (1)$$

DR (dose ratio) is the ratio of the $EC_{50}$ of α-MSH in the presence and the absence of antagonist. $pA_2$ is the negative logarithm of the concentration of antagonist which causes a dose ratio of 2, i.e., the $EC_{50}$ in the presence of antagonist is twice the value of the $EC_{50}$ in the absence of antagonist. $IC_{50}$, $EC_{50}$, and $pA_2$ values are given as mean ± S.D. from at least 3 independent experiments unless otherwise stated.

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