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Discovery of Antitrypanosomal Indolylacetamides by a Deconstruction–Optimization Strategy Applied to Paullones

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The parasitic kinetoplastid diseases Leishmaniasis, Chagas disease and Human African Trypanosomiasis constitute serious threats for populations throughout the (sub-)tropics. Most available drugs to treat these diseases possess inadequate properties and candidates to fill the drug pipeline are urgently needed. Paullone-N^5-acetamides inhibit trypanothione synthetase (TryS), an essential kinetoplastid enzyme, and exhibit antiparasitic activity in the low micromolar range, but lack the desired selectivity against mammalian cells (selectivity index (SI): <10). With the aim to identify the paullones' moieties responsible for TryS inhibition and bioactivity, we applied molecular simplification and ring disconnection approaches. The new indolylacetamides lost activity against the expected molecular target (TryS) compared to the reference paullone MOL2008 (*Leishmania infantum* TryS IC_{50}: 150 nM; *Trypanosoma brucei* bloodstream form EC_{50}: 4.3 μM and SI: 2.4). However, several of them retained potency (*T. b. brucei* EC_{50}: 2.4–12.0 μM) and improved selectivity (SI: 5 to >25).

Introduction

Designated by the World Health Organization, the neglected tropical diseases (NTD) embody twenty infectious diseases wreaking havoc throughout impoverished communities in the developing world.[1] NTD have in common their devastating socio-economic impact not only through premature death but also through disabling morbidities. Thus, the diseases present a serious burden for affected health systems, especially in poorly equipped rural areas where risk of infection is looming.

Included in the NTDs are three kinetoplastid blood-borne infections.[2] The parasites are closely related unicellular, flagellated protozoans that are transmitted by blood-feeding insect vectors.[3] *Trypanosoma cruzi* is the causative agent of American Trypanosomiasis (Chagas disease) in Middle and South America, *T. brucei rhodesiense* and *T. b. gambiense* account for Human African Trypanosomiasis (Sleeping sickness) endemic in sub-Saharan regions.[4] *Leishmania spp.* are responsible for the different forms of Leishmaniasis encountered in subtropical and tropical regions worldwide.[5] If untreated, these diseases are either fatal or frequently lead to diverse chronic health conditions, e.g. bacterial superinfections, disfigurations or cardiomyopathies. Moreover, *T. b. brucei*, which is used as a laboratory model organism, threatens the livelihood of farmers by infesting cattle in some regions of Africa (Nagana disease).[6] The parasites appear in heterogenous stages in insect vectors and mammalian hosts: *T. cruzi* and *Leishmania spp.* have extra- and intracellular forms, while *T. brucei* is an extracellular parasite.[7]

Disease prevention by vaccination is still not possible, and the development of chemotherapies for Chagas disease and Leishmaniasis have not progressed for decades.[8] Most of the approved antitrypanosomal and antileishmanial drugs possess several drawbacks, e.g. severe adverse effects, toxicities, disease stage-dependent efficacy and high costs. Intravenous administration constitutes a widespread obstacle, and only a few drugs are available in preferable easy-to-handle oral forms with respect to the living conditions of affected populations. Emerging resistances against frequently used drugs further aggravate successful treatment.[9] As a result, the need for new effective, safe and orally-applicable drugs with novel modes of action remains urgent to date.[8,10]

Herein we report the identification of new antitrypanosomal compounds by structural simplification and ring disconnection of antiparasitic paullone-N^5-acetamides with the goal to improve drug properties and selectivity.

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Results and Discussion

Drug Design: Simplification of Paullone-N^5-acetamides

Paullone-N^5-acetamides are inhibitors of trypanothione synthetase-amidase (TryS), a key enzyme involved in redox homeostasis of *Trypanosoma* and *Leishmania*.[11–13] Trypanothione ($T[SH]_2$) is absent in mammalian hosts, rendering the trypanothione biosynthesis enzymes and redox pathways attractive targets for drug design.[14] $T[SH]_2$ is a dimer of glutathione (GSH) linked by spermidine (Spd), a ubiquitous polyamine (Figure 1).[15] TryS has been validated as drug target by genetic techniques and by chemical inhibition with small molecules.[16]

As paullones are ATP-competitive kinase inhibitors, antiprotozoal paullone-N^5-acetamides were assumed to similarly occupy the ATP binding pocket adjacent to the GSH and Spd binding sites in the synthetase domain of TryS.[17] However, in recent years this hypothesis was challenged, because the most potent paullone TryS inhibitors bind with higher affinity in the presence of ATP-mimicking nucleotides.[13]

To date, co-crystal structures of TryS with any inhibitor are unavailable and the exact binding mode of paullones in TryS enzymes remains ambiguous. However, for MOL2008 (Figure 1) kinetic analysis and isothermal titration calorimetry support a mechanism of inhibition competitive with Spd and uncompetitive with ATP and GSH.[13] In line with this experimental data, structural modelling suggested that the *N*-(aminoalkyl)-acetamide chain of this compound occupies the Spd binding site of TryS whereas the tetracyclic paullone scaffold is anchored by π-stacking with a phenylalanine residue. The paullone partial structure also exerts a steric effect on the second GSH-binding site of the enzyme, which during the second ligation step accommodates the GSH moiety of the glutathionylspermidine formed in the first step.[13] MOL2008 inhibits TryS of different trypanosomatid species with varying efficacy, demonstrating its strongest inhibitory effects against *Leishmania infantum* TryS (IC_{50}: 150 nM) while its anti-*Leishmania* activity is only modest (EC_{50}: 12.6 μM). Vice versa, a 3-fold higher anti-*T. b. brucei* activity of MOL2008 (EC_{50}: 4.3 μM) is observed, despite its weak inhibitory efficacy on the *Tb*TryS enzyme (59% at 30 μM).[12] These apparent conflictive findings suggest that other targets than *Tb*TryS are responsible for the antiparasitic activity of paullone-N^5-acetamides in *T. b. brucei*.

Moreover, MOL2008 shows a poor selectivity index (SI: 2.4) as determined from murine macrophage CC_{50} (J774 cell line) and *T. b. brucei* EC_{50}.[12] In another recent study, the high toxicity of MOL2008 on mouse macrophages was confirmed (CC_{50}: 10.3 μM).[13] These selectivity issues emphasize the need for attaining higher potency against the parasites and/or lower cytotoxicity against mammalian cells. This goal is probably difficult to achieve with the unmodified paullone scaffold and thus substantial structural modifications are communicated here.

Numerous literature examples reported highly effective structural simplification campaigns, especially for complex polycyclic annulated ring systems in natural products.[18] The fragmentation can have multiple effects on compound properties, both good and bad, e.g. on binding mode, solubility or off-target effects. Similar hit deconstruction and subsequent optimization studies previously resulted in the discovery of marketed drugs, e.g. the kinase inhibitors dabrafenib and tofacitinib.[19]

Paullones consist of a 2-phenylindole motif (rings A, B and C) bridged by an acetamide moiety to form an azepinone ring (ring D, Figure 2). In the absence of a co-crystal structure supporting structure-based drug design, we performed a trial-and-error structural simplification approach to increase the fraction of sp^3-hybridized carbons (Fsp^3) of the new compounds compared to the paullone parent structure. Increasing the Fsp^3

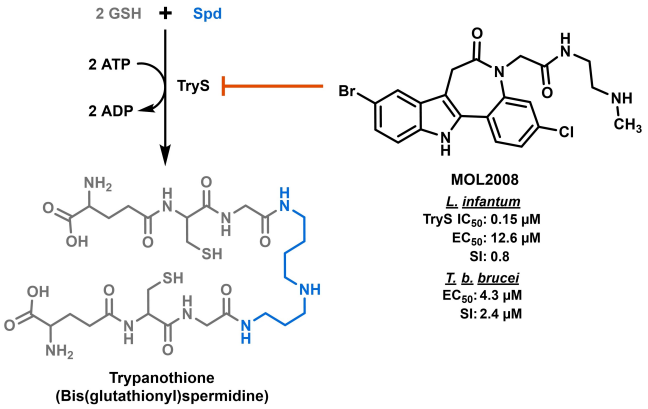


Figure 1. Trypanothione synthetase (TryS), at expenses of ATP, conjugates two glutathione molecules (grey) to spermidine (blue) to form trypanothione. MOL2008 is one of the most potent TryS inhibitors, carrying an aminoalkyl residue. Values taken from.[12] SI: Selectivity index calculated as the ratio of CC_{50} for murine macrophages (cell line J774) vs. EC_{50} for *L. infantum* or *T. b. brucei* parasites.

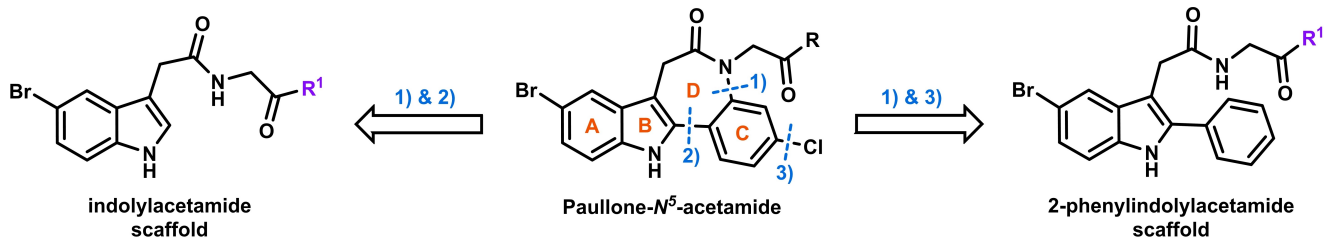


Figure 2. Structural simplification of paullone-N^5-acetamides led to indolylacetamide and 2-phenylindolylacetamides scaffolds which were substituted with various amines (R^1).

is a frequently and successfully applied method to improve drug-likeness.[20]

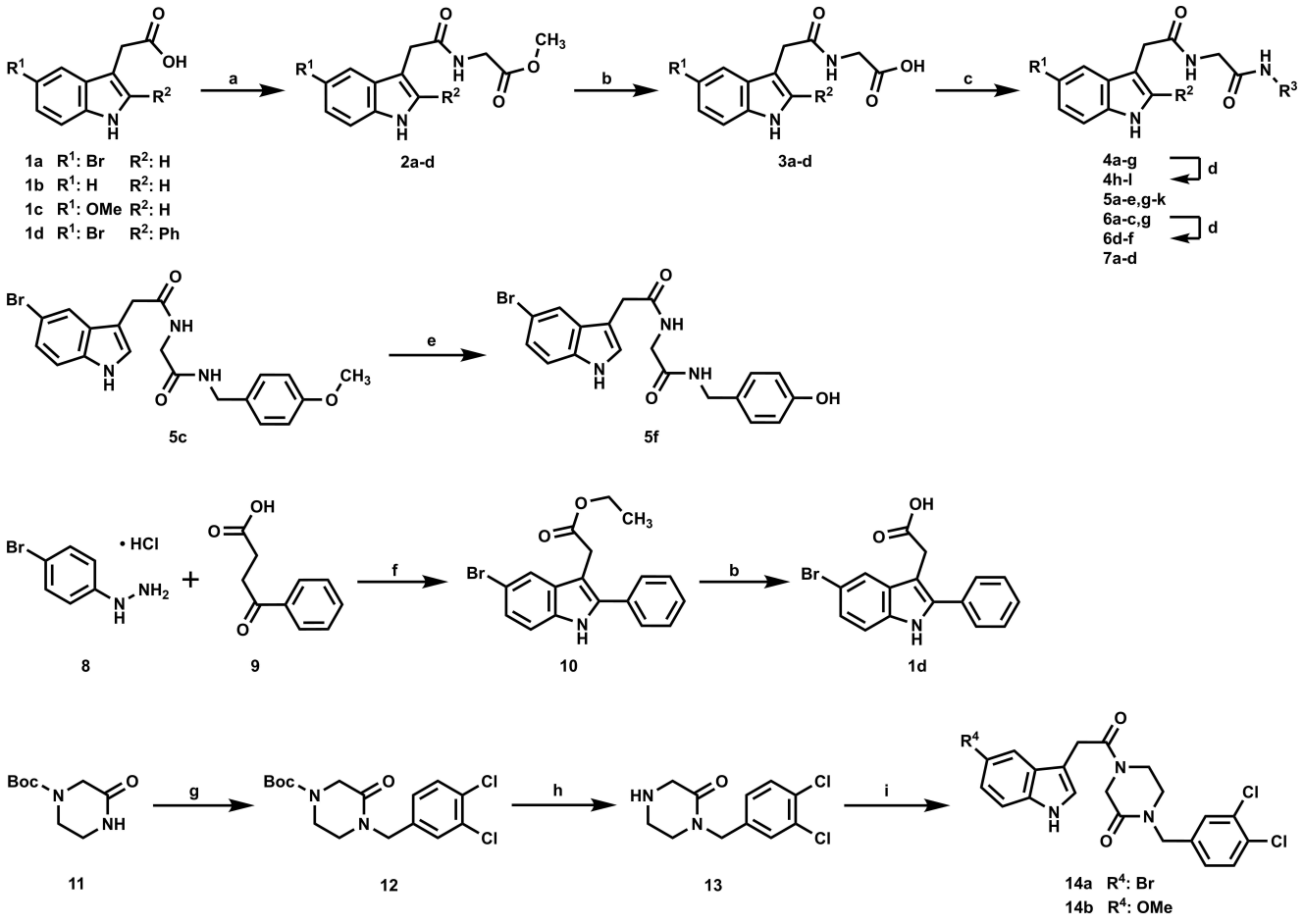
In this work, we truncated the paullones' benzene ring (ring C), which concomitantly disrupted the azepinone ring, resulting in an indolylacetamide scaffold (Figure 2). Additionally, by disconnecting only the *N*-aryl bond of the azepinone and omission of the chlorine substituent, a 2-phenylindolylacetamide structure was designed. This modification was made to elucidate the contribution of ring C to antitrypanosomal activity and selectivity.

Severing the azepinone ring leads to higher molecular flexibility and adds an additional hydrogen bond donor in both new scaffolds. Although the bromine of the indole part of paullone-N^5-acetamides like MOL2008 contributes to lipophilicity, we decided to retain this substituent in our initial attempts because earlier studies revealed its contribution to TryS binding.[21] The two new scaffolds were substituted with several side chains that had previously been identified as active moieties on paullones.[11,13,21]

The goal of this study was to determine the contribution of the tetracyclic scaffold of paullone-N^5-acetamides to anti-TryS activity and to improve the selectivity of the new compounds towards infective *T. b. brucei* with regard to MOL2008 by deconvoluting the paullone structure. The new derivatives were tested against recombinant forms of TryS from *L. infantum* and *T. brucei*, and against bloodstream *T. b. brucei* parasites and murine macrophages (i.e. physiological relevance in infection control).

Chemistry

The procedures for the syntheses of the title compounds are depicted in Scheme 1. For products **4**–**7** the synthetic routes started from appropriately substituted (**1a**,**c**–**d**) or unsubstituted (**1b**) 1*H*-indole-3-ylacetic acids, respectively, which were successively coupled with methyl glycinate to yield amide esters **2a**–**d** and deprotected with aqueous sodium hydroxide. The resulting acids **3a**–**d** were coupled with amines to yield the final products or Boc-protected precursors (**4a**–**g**, **5a**–**e**, **g**–**k**, **6a**–**c**, **g**, **7a**–**d**). Because deprotection in diethyl ether yielded oily products, Boc-protected compounds (**4b**–**d**, **g**, **6a**–**c**) were deprotected with hydrogen chloride in cyclopentyl methyl ether to yield the corresponding amino hydrochlorides (**4i**–**l**,



Scheme 1. Reagents and conditions: (a) GlyOMe·HCl, EDC·HCl, DIPEA, CH_2Cl_2, rt, 18–92 h, 54–97%; (b) 2 M NaOH, MeOH or EtOH, rt, overnight, 41%-quant.; (c) amine, EDC·HCl, DIPEA, CH_2Cl_2 or MeCN, rt, 17–72 h, 15–86% (for residues R^1 to R^3 refer to Tables 1, 2, 3 and 4); (d) 3 M HCl in CPME, CH_2Cl_2 or 1,4-dioxane, rt, 24 h, 93%-quant.; (e) 1. BBr_3, CH_2Cl_2, rt, 4–22 h, 2. H_2O, rt, 1–2 h, 58–94%; (f) H_2SO_4, EtOH, 130 °C, 8–14 h, 10–51%; (g) 1. NaH, DMF, N_2, 0 °C, 30 min, 2. 3,4-dichlorobenzyl bromide, rt, 3 h, 75%; (h) TFA, CH_2Cl_2, rt, 17.5 h, 91%; (i) **1a** or **1c**, EDC·HCl, DIPEA, CH_2Cl_2, rt, 48 h, 62–82%.

6d–**f**).[22] The methyl ether of **5c** was cleaved with boron tribromide in dichloromethane to give the phenol **5f**.

We gained access to the non-commercial 2-(5-bromo-2-phenyl-1*H*-indol-3-yl)acetic acid core of **1d** via a Fischer indole synthesis from 4-bromophenylhydrazine hydrochloride (**8**) and 3-benzoylpropionic acid (**9**) in ethanol, yielding moderate amounts of ethyl ester **10**. Deprotection of **10** gave the acid **1d** which was processed to the final products through the coupling-deprotection-coupling sequence described above.

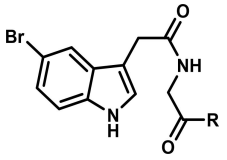
The synthesis of the cyclic bisamides **14** started with deprotonation of 1-Boc-3-oxopiperazine **11** in the presence of sodium hydride and alkylation with 3,4-dichlorobenzyl bromide, producing **12**. The Boc-group of **12** was removed by trifluoroacetic acid in dichloromethane and the resulting amine **13** was coupled with respective indoleacetic acids (**1a** or **1c**) to afford the final products (**14a**–**b**).

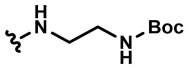
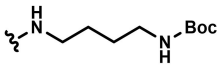
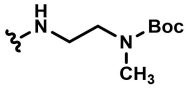
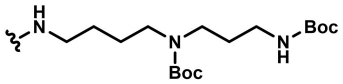
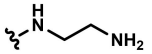
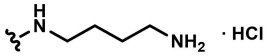
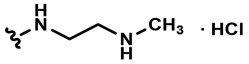
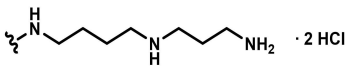
Biochemistry

As discussed above, several paullones presenting alkyl chains of different lengths and with polar substituents proved to be among the most potent inhibitors described so far for *Li*TryS with nanomolar IC_{50} values.[12,13] However, the relevance of the paullone architecture for target recognition remained elusive. We therefore screened all new compounds against *Li*TryS at a fixed concentration of 30 μM and at almost physiological concentrations of the enzyme substrates. Under these conditions, most compounds did not affect the activity of *Li*TryS at all (Supporting Information, Table S1). For example, compounds **4j**, **4l** and **6e** (structures in Table 1, Figure S1, and Table 3, respectively), which represent the analogues of MOL2008 and of a derivative with a piperazine substituent (*Li*TryS IC_{50}: 0.3 μM, referred to as *'compound 20'* in [13]), proved completely inactive towards this enzyme. These results demonstrate that disruption of the paullones' tetracyclic scaffold is highly detrimental for TryS inhibition and suggest that its 6,7,5,6-annulated ring structure is critical for binding to this specific molecular target.

Furthermore, our current evidence suggests that *Tb*TryS is not a target of inhibition by paullones because the residue motif and conformation of a loop that modulates the accessibility of substrates (Spd and GSH) to the enzyme active site differs between trypanosomal and leishmanial TryS.[13] This steric restriction, but not specific protein-ligand interactions, may eventually be overcome by analogues with larger flexibility, a requirement fulfilled by several of the compounds prepared in the present study. This hypothesis was then tested by assaying all new compounds (30 μM) against *Tb*TryS. As

Table 1. Biological activity of indolylacetamide derivatives with aminoalkyl substituents.



Cmpd	R	*T. b. brucei* bloodstream form Viability [%] (10 μM)	EC_{50} [μM]	Mouse macrophages (cell line J774) Viability [%][a]	CC_{50} [μM]
4a		98.5 ± 8.1	n.d.	n.d.	n.d.
4b		131.5 ± 4.9	n.d.	n.d.	n.d.
4c		119.2 ± 3.4	n.d.	n.d.	n.d.
4d		9.9 ± 5.5	8.4 ± 1.1	−0.2 ± 1.0 [85 μM]	46.1 ± 1.0
4h		103.7 ± 3.5	n.d.	n.d.	n.d.
4i		121.5 ± 4.3	n.d.	n.d.	n.d.
4j		96.4 ± 5.0	n.d.	n.d.	n.d.
4k		113.8 ± 11.5	n.d.	n.d.	n.d.
Nifurtimox		58–65 % [5 μM]	5.3 ± 0.4[b]	n.d.	140.0 ± 2.0[b]

[a] Triton X-100 0.1 % resulted in 0 % viability upon 24 h incubation. [b] EC_{50} and CC_{50} values for Nfx were determined under the same conditions as for the compounds investigated herein and reported previously from our laboratory.[23,24] N.d.: not determined; SD is expressed as σ·n−1 (%).

shown in the Supporting Information (Table S1), none of the compounds affected *Tb*TryS activity to a significant level. Only the indolyl- or 2-phenylindolylacetamide derivatives containing the longest alkyl chain with free amines resembling the native substrate Spd (**4k** and **6f**, respectively) were capable to inhibit *Tb*TryS with a similar potency (52–62% inhibition at 30 μM) to that of MOL2008 (IC_{50}: 30 μM). Altogether, these results confirm that specific interactions between Spd-like substituents are relevant for binding paullone or related indole-based compounds to *Tb*TryS active site.

Biology

In addition to the assays against both TryS enzymes, all synthesized compounds were initially tested in a phenotypic viability assay at 10 μM against *T. b. brucei* bloodstream forms.[25] EC_{50} values were determined for compounds with more than 50% viability inhibition. For compounds exhibiting considerable antiparasitic activity, toxicity on mouse macrophages (cell line J774) was determined at approximately the tenfold concentration of the determined EC_{50} for an overview of selectivity. CC_{50} values were determined if an appropriate level of selectivity was expected based on the results of the single-dose toxicity tests. Nifurtimox (Nfx) was used as positive control in the parasite viability assays and Triton X-100 0.1% was used as a cytotoxic agent in the macrophage viability assays.

Biological Evaluation

Results of indolylacetamides with aminoalkyl substituents

Results for indolylacetamides bearing aminoalkyl substituents are shown in Table 1. Compound design was related to known antitrypanosomal paullones, e.g. **4j** carries the substituent of MOL2008.[13] The synthesized amines vary in alkyl linker length and substitution pattern. No antitrypanosomal activity was expected for Boc-protected precursors (compounds **4a–d**), but surprisingly extensive parasite death was detected in the case of the Boc-protected spermidine derivative **4d**. While a single-digit micromolar EC_{50} was determined (8.4 μM), the derivative turned out to be cytotoxic against mammalian cells (CC_{50}: 46.1 μM).

Except for **4d**, the majority of the derivatives in series **4** did not reduce parasite survival, although paullone amines such as MOL2008 have a phenotypic effect against *T. b. brucei* parasites. This might be due to the high number (≥ 3) of hydrogen bond donors in the compounds of series **4**, which correlates with low permeability and might thereby prohibit intracellular enrichment.[26] A similar phenotypic activity pattern was previously reported for a related Boc-protected spermidine derivative against trypanosomatids, and the corresponding unprotected amine was also inactive.[27] Boc-groups presumably contribute to an increased membrane permeability due to higher lipophilicity of the resulting compounds. Clearly, this concept cannot be generalized for compounds **4** since only for one (**4d–4k**) of several (**4a–4h**, **4b–4i**, **4c–4j** and **4g–4l**) pairs of analogues this modification contributed significantly to bioactivity (Table 1 and Supporting Information, Table S1).

Results of indolylacetamides with benzylamide substituents

Paullone-N^5-acetamides with terminal benzyl substituents were previously reported to be potent inhibitors of *T. b. brucei* proliferation.[11] Therefore, this structural feature was also introduced in the side chains of the indolylacetamides reported here, leading to series **5** as presented in Table 2.

In this context, decoration of the phenyl ring was modified by means of a Topliss approach.[28] Although this method has been introduced several decades ago, it still constitutes a reasonable strategy for drug optimization, enabling rapid structure-activity relationship exploration in phenyl-substituted compounds even without knowledge about the target structure.[29] Iterative comparisons of the biological activities of two phenyl substituents lead to the next proposed substituent and ultimately to a suitable phenyl substitution pattern.[28]

We collected biological data of the five compounds in the three upper levels in the decision tree in Figure 3 (**5a–e**). A significant improvement was observed for the 3,4-di-Cl compound **5e** (EC_{50}: 12.0 μM), indicating the favorable influence of lipophilic electron-withdrawing substituents on the antiparasitic activity. Compounds **5a–d** did not trigger a reduction of parasite survival, except for the weak activity of **5d** (4-CH_3). However, **5d** proved to be slightly toxic for mammalian cells at 30 μM (Table 2). To explore our hypothesis, that the right branch would lead to higher activity, we synthesized more compounds from the decision tree depicted in Figure 3. Two of them (**5f**, **5g**) were selected to confirm that the left and middle branches of the Topliss tree would not lead to activity and, indeed, they met our expectations (Figure 3). Additionally, following up on **5e**, two compounds (**5h**, **5i**) were chosen to

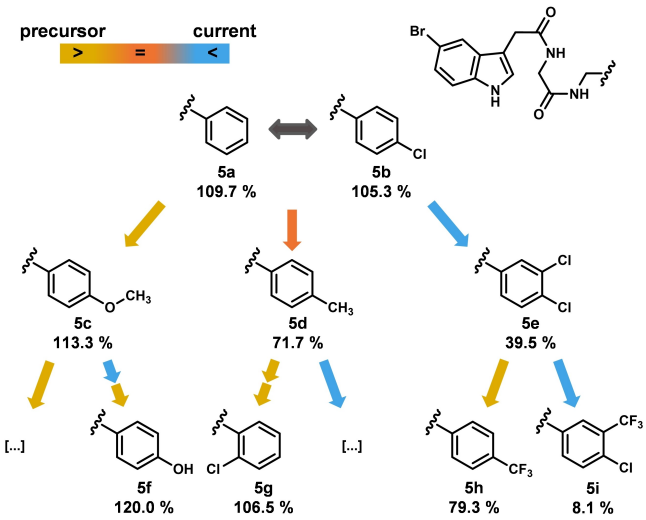
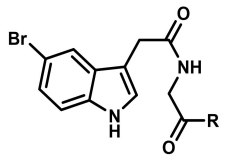
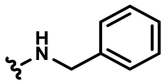
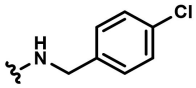
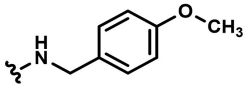
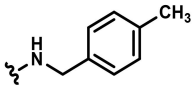
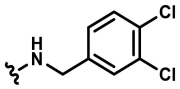
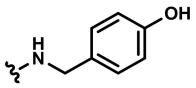
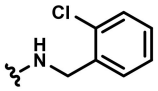
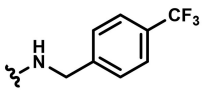
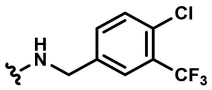


Figure 3. Arrangement of benzylamide derivatives **5** in the Topliss decision tree. Values reflect parasite viability at a concentration of 10 μM.

Table 2. Biological results of indolylacetamide derivatives with benzylamide substituents.



Cmpd	R	*T. b. brucei* bloodstream form Viability [%] (10 μM)	EC_{50} [μM]	Mouse macrophages (cell line J774) Viability [%][a]	CC_{50} [μM]
5a		109.7 ± 5.1	n.d.	96.5 ± 4.4 [30 μM]	n.d.
5b		105.3 ± 16.3	n.d.	103.3 ± 4.2 [30 μM]	n.d.
5c		113.3 ± 6.7	n.d.	102.1 ± 5.0 [30 μM]	n.d.
5d		71.7 ± 2.7	n.d.	69.6 ± 9.8 [30 μM]	n.d.
5e		39.5 ± 10.7	12.0 ± 0.0	99.2 ± 2.8 [30 μM] 16.6 ± 6.8 [100 μM]	Compound precipitation at > 100 μM
5f		120.0 ± 9.2	n.d.	98.2 ± 1.4 [30 μM]	n.d.
5g		106.5 ± 6.4	n.d.	95.1 ± 2.5 [30 μM]	n.d.
5h		79.3 ± 7.5	n.d.	102.3 ± 3.6 [30 μM]	n.d.
5i		8.1 ± 2.7	9.8 ± 1.0	0.2 ± 1.2 [100 μM]	53.0 ± 1.2
Nifurtimox		58–65 % [5 μM]	5.3 ± 0.4[b]	n.d.	140.0 ± 2.0[b]

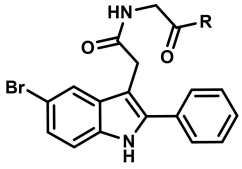
[a] Triton X-100 0.1 % resulted in 0 % viability upon 24 h incubation. [b] EC_{50} and CC_{50} values for Nfx were determined under the same conditions as for the compounds investigated herein and reported previously from our laboratory.[23,24] N.d.: not determined; SD is expressed as σ·n−1 (%).

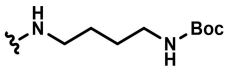
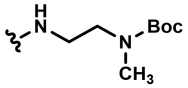
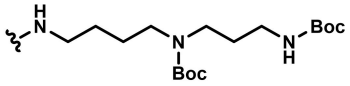
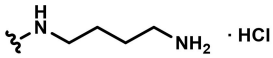
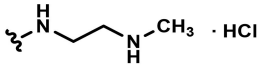
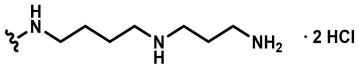
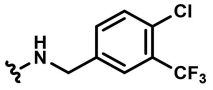
potentially improve antitrypanosomal activity. The 4-CF_3 substituent (**5h**) did retain weak activity, although it reduced parasite survival less than **5i** did. This is in line with the construction of the Topliss tree, ranking **5h** between the 4-Cl and 3,4-di-Cl compounds. The 4-Cl,3-CF_3-substituted compound **5i** triggered nearly complete parasite death at 10 μM and the EC_{50} against *T. b. brucei* was determined (9.8 μM), but **5i** proved to be rather cytotoxic for mouse macrophages (CC_{50}: 53.0 μM). In contrast, its 3,4-dichloro-substituted analogue **5e** was less cytotoxic (Table 2).

Results of 2-phenylindolylacetamides

Some of the 2-phenylindole derivatives of series **6** (Table 3) exhibited considerably improved antiparasitic activity and selectivity. For example, the lipophilic benzylamide derivative **6g** (*Tb* EC_{50}: 4.0 μM) showed substantial antiproliferative activity that is approximately two-fold lower than its counterpart **5i** which lacks the 2-phenyl substitution of the indole. A similar potency enhancement was observed for congeners **6a**–**c** which all showed improved antiparasitic activity compared to the respective analogues **4b**–**d**. At variance with series **4**, the Boc-protection of basic amino side chains of members of series **6** contributed markedly to increase bioactivity of all three Boc-protected compounds against *T. b. brucei*. Along these lines, **6a**–**c** inhibited parasite growth, whereas the deprotected amines **6d**–**f** failed to do so. Moreover, the fact that **6a** and its analogue **6c** are equipotent suggests that the Boc-aminopropyl terminus of the latter is redundant for antiproliferative activity. However, the additional aminopropyl group in **6c** reduced

Table 3. Biological activity of derivatives **6** based on a 2-phenylindole parent scaffold.



Cmpd	R	*T. b. brucei* bloodstream form Viability [%] (10 μM)	EC_{50} [μM]	Mouse macrophages (cell line J774) Viability [%][a]	CC_{50} [μM]
6a		1.4 ± 0.9	2.6 ± 1.2	54.1 ± 3.2 [30 μM]	Compound precipitation at > 30 μM
6b		6.2 ± 5.3	8.2 ± 1.0	23.8 ± 3.8 [85 μM]	39.8 ± 0.8
6c		−0.1 ± 1.5	2.4 ± 1.0	38.8 ± 7.0 [100 μM]	60–100
6d		83.5 ± 14.4	n.d.	n.d.	n.d.
6e		109.4 ± 3.2	n.d.	n.d	n.d
6f		87.2 ± 14.1	n.d.	n.d.	n.d.
6g		−0.6 ± 1.0	4.0 ± 1.1	24.3 ± 9.0 [40 μM]	Compound precipitation at > 25 μM
Nifurtimox		58–65 % [5 μM]	5.3 ± 0.4[b]	n.d.	140.0 ± 2.0[b]

[a] Triton X-100 0.1 % resulted in 0 % viability upon 24 h incubation. [b] EC_{50} and CC_{50} values for Nfx were determined under the same conditions as for the compounds investigated herein and reported previously from our laboratory.[23,24] N.d.: not determined; SD is expressed as σ·n−1 (%).

cytotoxicity and, concomitantly, improved its selectivity index (discussed below; see Table 5).

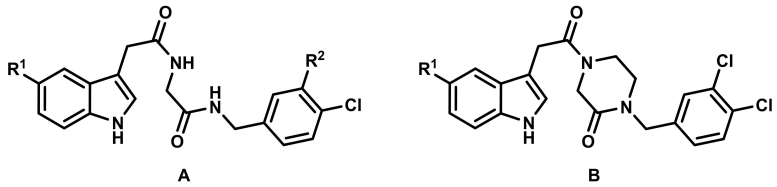
Investigation of indole 5-substitution and chain rigidification

The benzylamides **5e** and **5i** are new antitrypanosomal compounds with a hitherto unknown biological target. We further explored their SAR by modifying the parts retained during structural simplification. Therefore, the 5-bromo substituent on the indole was deleted or replaced for a methoxy group. The methoxy group was chosen to alter the electrostatic properties of the indole and introduce another hydrogen bond acceptor while retaining a substituent to fill a potential pocket. According to the literature, several compounds with 5-methoxyindole moieties possess anti-trypanosomal activity.[30] Examination of the established matched molecular series[31] led to the conclusion that the 5-bromo substituent is indispensable for activity as none of the four prepared derivatives (**7a**–**d**) reduced parasite viability. One could speculate that the bromo substituent at this position forms a halogen bond to the as yet unidentified target or fills a suitable binding pocket.[32]

None of the free amines of series **4** and **6** showed cellular effects which could be due to permeability issues. The number of rotatable bonds and hydrogen bond donors has been associated with negative impacts on permeability and both parameter counts are high in the aminoalkyl derivatives.[26,33,34] Additionally, amides are prone to attach water molecules and the resulting desolvation penalty may prohibit adequate membrane penetration.[33]

To tackle both of these potential weaknesses, a cyclization of the bisamide chain of **5e** with an ethylene bridge was implemented, resulting in the 2-oxopiperazines **14a** and **14b**. Indeed, the bromo-substituted 2-oxopiperazine **14a** exhibited an improved *T. b. brucei* EC_{50} of 5.6 μM (Table 4) compared to 12.0 μM from its linear analogue **5e**. Moreover, the exchange of the 5-bromo substituent for a methoxy group followed the SAR pattern observed with previous matched pairs, as the 5-methoxy analogue **14b** was clearly inferior to **14a** regarding antiparasitic activity. Displaying a single-digit antitrypanosomal activity and a selectivity index of 9.4, **14a** is a promising starting point for further optimization of this new class of antiparasitic compounds considering its reduced rotatable bond and hydrogen bond donor counts.

Table 4. Biological activity of modified indolylamides with benzylamide substituents.



A B

Cmpd	Scaffold	R^1	R^2	*T. b. brucei* bloodstream form Viability [%] (10 μM)	*T. b. brucei* bloodstream form EC_{50} [μM]	Mouse macrophages (cell line J774) Viability [%][a]	Mouse macrophages (cell line J774) CC_{50} [μM]
7a	A	H	Cl	97.9 ± 15.8	n.d.	n.d.	n.d.
7b	A	H	CF_3	93.9 ± 2.1	n.d.	n.d.	n.d.
7c	A	OMe	Cl	98.9 ± 1.8	n.d.	n.d.	n.d.
7d	A	OMe	CF_3	96.0 ± 8.2	n.d.	n.d.	n.d.
14a	B	Br	–	4.3 ± 2.0	5.6 ± 0.9	52.5 ± 2.1 [60 μM]	52.9 ± 5.7
14b	B	OMe	–	78.4 ± 0.9	n.d.	n.d.	n.d.
Nifurtimox				58–65% [5 μM]	5.3 ± 0.4[b]	n.d.	140.0 ± 2.0[b]

[a] Triton X-100 0.1% resulted in 0% viability upon 24 h incubation. [b] EC_{50} and CC_{50} values for Nfx were determined under the same conditions as for the compounds investigated herein and reported previously from our laboratory.[23,24] N.d.: not determined; SD is expressed as $\sigma \cdot n-1$ (%).

Selectivity of the most promising compounds

In the course of our studies we discovered new antitrypanosomal compounds for which we determined EC_{50} values against *T. b. brucei* and CC_{50} values against murine macrophages. The results for the most promising compounds are summarized in Table 5. Compared to the parent compound MOL2008 (CC_{50}: 10.3 μM, SI: 2.4), and tested under identical conditions, all of the new hits showed lower cytotoxicity against macrophages. A comparable or higher antitrypanosomal activity for some of the derivatives (**6a**, **6c**, **6g**, **14a**) concomitantly led to an improved selectivity. The molecule with the highest selectivity turned out to be **6c** (CC_{50}: >60 μM, SI: >25).

Conclusion

New therapeutics to treat protozoan neglected diseases are urgently needed. We performed formal molecular simplification and ring disconnection of paullones with the aim of optimizing their bioactivity. These approaches led to indolylacetamide derivatives with antiparasitic activity against *T. b. brucei* parasites in single-digit micromolar concentrations and improved selectivity compared to the template paullone MOL2008. Structure-activity relationship studies revealed that a 5-bromo substituent on the indole is essential and that a 2-phenyl substituent is favorable for antitrypanosomal activity. While compounds with free amino groups in the side chain turned out to be inactive, several analogues with Boc-protected amino groups displayed antiparasitic activity. Applying a Topliss approach, we discovered potent benzylamide derivatives. The bisamide motif of these benzylamide-substituted derivatives was cyclized to a 2-oxopiperazine with an increase in activity. The phenotypic activity data obtained *via* the Topliss approach (**5e**, **5i**), the results of the 2-phenylindoles (**6a**–**c**, **6g**) and the activity improvement by masking the hydrogen bond donors in the amide chain (**14a**) all suggest that lipophilicity is a crucial contributor to activity for the series presented herein. Alternative simplification approaches around the paullone core are currently under evaluation and will be reported in due time.

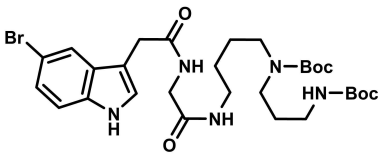
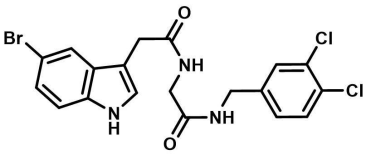
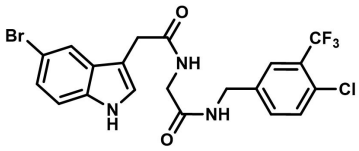
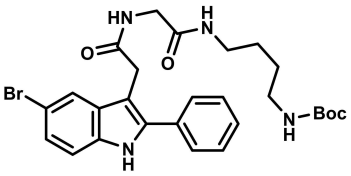
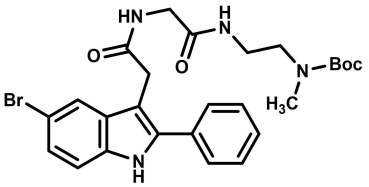
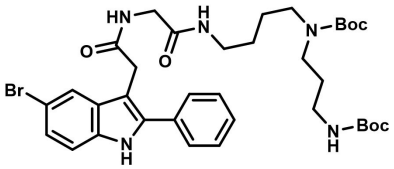
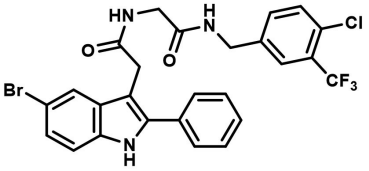
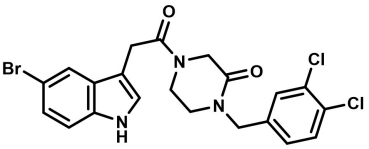
Experimental Section

Chemistry

Materials and Methods

Starting materials and reagents were purchased from abcr GmbH (Karlsruhe, Germany), Acros Organics B.V.B.A. (Geel, Belgium), Alfa Aesar (Kandel, Germany), BLDpharm Ltd. (Shanghai, China or GmbH, Kaiserslautern, Germany), Enamine (Kyiv, Ukraine), Fluorochem Ltd. (Derbyshire, UK) and Sigma-Aldrich (Steinheim, Germany). All reagents and solvents were used without further purification. *N*,*N*-dimethylformamide was purchased in Uvasol® quality and dichloromethane was dried according to published procedures.[35] Water was demineralised in-house before use. Solvents in HPLC quality were purchased from Sigma-Aldrich or Acros Organics and water for HPLC was bidestilled in-house. Deuterated solvents for NMR spectroscopy were purchased from Deutero GmbH (Kastellaun, Germany). Manufacturers of chemicals and instruments for the biological assays are indicated in the experimental procedures. Reaction monitoring was performed using thin layer chromatography (TLC): Polygram SIL G/UV254, 0.2 mm silica gel 60, 40×80 mm (Macherey-Nagel, Düren, Germany), visualization by UV light (254 nm, 366 nm) in a UV cabinet (CAMAG, Muttenz, Switzerland). Melting points (m.p.) were measured in open glass capillaries on an electric Electrothermal IA9200 variable heater (Cole-Parmer Instrument Company Ltd., St Neots, UK). Decomposition is indicated as (decomp.). Infrared spectra were recorded on a Nicolet FT-IR 200 spectrometer (Thermo Nicolet, Madison, WI, USA) using KBr pellets. ^{1}H NMR spectra and ^{13}C NMR spectra were recorded on Bruker Avance III 400, Bruker Avance II 600 or Bruker Avance III HD 500 spectrometers (Bruker Biospin, Rheinstetten, Germany) at the NMR laboratories of the Chemical Institutes at TU Braunschweig, Germany. Chemical shifts are

Table 5. Biological activity of the most promising compounds from this study.

Cmpd	Structure	*T. b. brucei* EC_{50} [μM]	Macrophage CC_{50} [μM]	Selectivity index
4d		8.4 ± 1.1	46.1 ± 1.0	5.5
5e		12.0 ± 0.0	< 100	< 8
5i		9.8 ± 1.0	53.0 ± 1.2	5.4
6a		2.6 ± 1.2	~30	11.5
6b		8.2 ± 1.0	39.8 ± 0.8	4.8
6c		2.4 ± 1.0	> 60	> 25
6g		4.0 ± 1.1	< 40	< 10
14a		5.6 ± 0.9	52.9 ± 5.7	9.4
Nifurtimox		5.3 ± 0.4[a]	140.0 ± 2.0[a]	23[b]

[a] EC_{50} and CC_{50} values for Nfx were determined under the same conditions as for the compounds investigated herein and reported previously from our laboratory.[23,24] [b] The SI of Nfx was reported in.[24] SD is expressed as σ·n–1 (%); Selectivity indices (SI) were calculated as the ratio of CC_{50} for murine macrophages (cell line J774) vs. EC_{50} for *T. b. brucei* parasites.

reported as parts per million (ppm) relative to tetramethylsilane as internal standard (δ = 0 ppm). ^{13}C NMR signals were assigned based on results of ^{13}C-DEPT135 experiments. DMSO-d_6 was used as solvent for all NMR experiments. Elemental analyses (CHN) were performed in-house on a CE Instruments Flash EA® 1112 Elemental Analyzer (Thermo Quest, San Jose, CA, USA). Percentages for calculated (calc.) and found (found) values are indicated. Measurements were conducted in duplicates or triplicates and arithmetic means were calculated. Mass spectrometry (MS) was performed in-house on an ExpressionL CMS spectrometer (Advion, Ithaca, NY, USA) in positive and negative ionization modes. Carrier gas: Nitrogen gas. Direct injection of a methanolic solution (ca. 50 μg/

1.5 mL, injection volume: ca. 100–150 µL) Electron spray ionization (ESI): source gas temperature: 200 °C (pos. mode), 250 °C (neg. mode), capillary temperature: 250 °C, capillary voltage: 180 V, spray voltage: 3500 V. Atmospheric pressure chemical ionization (APCI) was used if ESI was unsuccessful: Source gas temperature: 250 °C (pos. mode), 350 °C (neg. mode), APCI corona discharge: 5.0 µA, capillary temperature: 250 °C, capillary voltage: 170 V (pos. mode), 180 V (neg. mode). Software: Advion Mass Express and Advion Data Express (Version 6.2.21). Purity (≥95 %) was determined using high performance liquid chromatography (HPLC) methods with isocratic or gradient elution. For compounds with poor absorption coefficients at A = 254 nm, the absorbance at A = 280 nm was used for purity assessment. The following HPLC devices and settings were used: Merck Hitachi Elite LaChrom system (Hitachi High Technologies Corporation, Tokyo, Japan); the threshold of the integration method was set to 1000; diode array detector (DAD): L-2450; pump: L-2130; autosampler: L-2200; organizer box: L-2000; column: LiChrospher® 100 RP-18 (5 µM) LiChroCART 125-4; guard column: LiChrospher® 100 RP-18 (5 µm) LiChroCART® 4-4. Merck Hitachi Elite LaChrom system (Hitachi High Technologies Corporation, Tokyo, Japan); the threshold of the integration method was set to 50; detector: L-2400; pump: L-2130; autosampler: L-2200; organizer box: L-2000; column: LiChrospher® 100 RP-18 (5 µM) LiChroCART 125-4; guard column: LiChrospher® 100 RP-18 (5 µm) LiChroCART® 4-4. Flow rate: 1.000 mL/min; detection wavelength: 254 nm and 280 nm (isocratic), 254 nm (gradient); AUC was assessed, 100 % method; t_{M+S}: retention time of compound, t_M: dead time related to DMSO. An acetonitrile/water mixture was used for gradient elution (0–2 min: 10 % MeCN; 2–12 min: 10 %–90 % MeCN (linear) 12–20 min: 90 % MeCN). For isocratic elution, acetonitrile/water or acetonitrile/aqueous buffer mixtures were used. The aqueous triethylamine/triethylammonium sulfate buffer (pH 2.7) was prepared by dissolving triethylamine (20 mL) and sodium hydroxide (242 mg) in bidestilled water (ad 1 L). The pH was adjusted to 2.7 by dropwise addition of concentrated H_2SO_4. Absorption maxima (λ_{max}) were extracted from the UV spectra recorded by the DAD detector in the peak maxima. If the elemental analyses were inconclusive, HR-MS was performed. Electron spray ionization (ESI) HR-MS spectra were recorded on LTQ-Orbitrap Velos (ThermoFisher Scientific, Bremen, Germany) at the MS laboratories of the Chemical Institutes at TU Braunschweig, Germany. Tetradecyltrimethylammonium bromide was used as an internal standard. Compounds were dissolved in methanol to obtain a concentration of approximately 50 µg/mL. Flow rate: 1 µL/min, spray voltage: pos. mode: 2.3–2.8 kV, neg. mode: 1.7–2.5 kV. Software: Xcalibur 2.2 (ThermoFisher Scientific, Waltham, MA, USA).

Syntheses

General Procedure A for amide couplings

The carboxylic acid (1 eq.), EDC·HCl (1.4 eq.), (HOBt (1.4 eq.)) and DIPEA (1.4 eq.; if hydrochlorides were used: 2.4 eq.) were suspended in dichloromethane, acetonitrile or *N*,*N*-dimethylformamide and the amine or its hydrochloride (1.4 eq.) were added. The mixture was layered with argon and stirred at room temperature for the indicated time. The solvent was evaporated under reduced pressure and the residue was purified by column chromatography with the solvent indicated for each compound to afford the product.

General Procedure B for Boc deprotection

The Boc-protected amine (50–100 mg) was dissolved or suspended in 1,4-dioxane or dichloromethane (3 mL). Hydrogen chloride (3 M in cyclopentyl methyl ether, 1 mL) was added. The mixture was stirred for one day at room temperature until TLC indicated complete conversion of the protected amine. The solvent was evaporated under reduced pressure to afford the product.

***tert*-Butyl (2-{2-[2-(5-bromo-1*H*-indole-3-yl)acetamido]acetamido}ethyl)carbamate (4 a)**. According to General Procedure A from *N*-[2-(5-bromo-1*H*-indole-3-yl)acetyl]glycine (**3 a**, 250 mg, 0.804 mmol), EDC·HCl (219 mg, 1.14 mmol), DIPEA (0.19 mL, 1.1 mmol) and *N*-(*tert*-butoxycarbonyl)ethylendiamine (175 µL, 1.11 mmol) in acetonitrile (2.5 mL) for 30 hours. Solvent for column chromatography: ethyl acetate/acetone (2 : 1). A pale yellow solid was obtained (199 mg, 0.440 mmol, 55 %). M.p.: 161–163 °C; IR (KBr): 3335 cm^{-1} (N–H), 3258 cm^{-1} (N–H), 1686 cm^{-1} (C=O), 1659 cm^{-1} (C=O), 1633 cm^{-1} (C=O); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.09 (s, 1H, indole NH), 8.11 (t, *J* = 5.8 Hz, 1H, amide NH), 7.89 (t, *J* = 5.7 Hz, 1H, amide NH), 7.75 (d, *J* = 1.9 Hz, 1H, ArH), 7.31 (d, *J* = 8.7 Hz, 1H, ArH), 7.28 (d, *J* = 2.4 Hz, 1H, ArH), 7.16 (dd, *J* = 8.5, 1.9 Hz, 1H, ArH), 6.81 (t, *J* = 5.6 Hz, 1H, carbamate NH), 3.65 (d, *J* = 5.8 Hz, 2H, –CH_2–), 3.55 (s, 2H, –CH_2–), 3.08 (q, *J* = 6.4 Hz, 2H, –CH_2–), 2.97 (q, *J* = 6.3 Hz, 2H, –CH_2–), 1.37 (s, 9H, –CH_3); ^{13}C NMR (126 MHz, DMSO) δ 28.2 (3C) (CH_3), 32.2, 38.8, 39.6 (overlapping with the DMSO signal), 42.1 (CH_2), 113.3, 121.2, 123.4, 125.6 (CH), 77.7, 108.7, 111.0, 129.1, 134.8, 155.6, 169.0, 170.8 (C); CHN : calc. C 50.34, H 5.56, N 12.36, found C 50.80, H 5.48, N 12.07; $C_{19}H_{25}BrN_4O_4$ (453.34); MS (ESI): m/z (%): pos.: 475.1 $[M+Na]^+$ (100) 419.0 [M-−33]$^+$, neg.: 451.0 $[M-H]^-$ (7), 376.9 [M-−76]$^-$ (100), 333.9 [M-118]$^-$ (20); HPLC (isocr.): 96.8 % at 254 nm, 98.9 % at 280 nm, t_{M+S} = 4.04 min, t_M(DMSO) = 1.12 min (MeCN/water = 40 : 60); HPLC (grad.) : 97.7 % at 254 nm, t_{M+S} = 9.39 min, t_M(DMSO) = 1.16 min; λ_{max} : 225 nm, 288 nm; HR-MS (ESI): m/z $[M+Na]^+$ calc. 475.09569, found 475.09601.

***tert*-Butyl-*N*-(4-{2-[2-(5-bromo-1*H*-indole-3-yl)acetamido]-acetamido}butyl)carbamate (4 b)**. According to General Procedure A from *N*-[2-(5-bromo-1*H*-indole-3-yl)acetyl]glycine (**3 a**, 120 mg, 0.386 mmol), EDC·HCl (104 mg, 0.543 mmol), DIPEA (0.10 mL, 0.57 mmol) and *N*-(*tert*-butoxycarbonyl)-1,4-diaminobutane (0.11 mL, 0.57 mmol) in acetonitrile (3 mL) for 68.5 hours. Solvent for column chromatography: ethyl acetate/ethanol (10 : 1). A colorless solid was obtained (112 mg, 0.232 mmol, 60 %). M.p.: 82–85 °C; IR (KBr) : 3304 cm^{-1} (N–H, br), 1655 cm^{-1} (C=O, br); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.08 (s, 1H, indole NH), 8.09 (t, *J* = 5.7 Hz, 1H, amide NH), 7.78–7.72 (m, 2H, amide NH, ArH), 7.31 (dd, *J* = 8.6, 0.5 Hz, 1H, ArH), 7.28 (d, *J* = 2.4 Hz, 1H, ArH), 7.16 (dd, *J* = 8.7, 2.0 Hz, 1H, ArH), 6.75 (t, *J* = 5.8 Hz, 1H, carbamate NH), 3.65 (d, *J* = 5.8 Hz, 2H, –CH_2–), 3.55 (d, *J* = 0.8 Hz, 2H, –CH_2–), 3.02 (q, *J* = 6.4 Hz, 2H, –CH_2–), 2.89 (m, 2H, –CH_2–), 1.37 (s, 9H, –CH_3), 1.35–1.31 (m, 4H, –CH_2–); ^{13}C NMR (126 MHz, DMSO) δ 28.3 (3C) (CH_3), 26.5, 26.9, 32.2, 38.3, 39.5 (overlapping with the DMSO signal), 42.1 (CH_2), 113.3, 121.1, 123.3, 125.6 (CH), 77.3, 108.7, 111.0, 129.1, 134.7, 155.6, 168.6, 170.7 (C); C21H29BrN4O4 (481.39); MS (ESI): m/z (%): pos.: 503.2 $[M+Na]^+$ (100), neg.: 479.2 $[M-H]^-$ (100), 405.1 [M-−75]$^-$ (95), 515.2 $[M+Cl]^-$ (14); HPLC (isocr.): 97.8 % at 254 nm, 98.6 % at 280 nm, t_{M+S} = 5.09 min, t_M(DMSO) = 1.11 min (MeCN/water = 40 : 60); HPLC (grad.): 97.6 % at 254 nm, t_{M+S} = 9.63 min, t_M(DMSO) = 1.14 min; λ_{max}: 226 nm, 288 nm; HR-MS (ESI): m/z $[M+Na]^+$ calc. 503.12644, found 503.12679.

***tert*-Butyl-*N*-(2-{2-[2-(5-bromo-1*H*-indole-3-yl)acetamido]-acetamido}ethyl)-*N*-(methyl)carbamate (4 c)**. According to General Procedure A from *N*-[2-(5-bromo-1*H*-indole-3-yl)acetyl]glycine (**3 a**, 134 mg, 0.431 mmol), EDC·HCl (116 mg, 0.605 mmol), DIPEA (0.11 mL, 0.60 mmol) and *N*-(*tert*-butoxycarbonyl)-*N*-methylethylenediamine (0.11 mL, 0.62 mmol) in acetonitrile (3 mL) for 66 hours. Solvent for column chromatography: ethyl acetate/ethanol (10 : 1). A colorless solid was obtained (138 mg, 0.296 mmol, 69 %). M.p.: 209 °C (decomp.); IR (KBr): 3299 cm^{-1} (N–H, br), 1667 cm^{-1} (C=O), 1640 cm^{-1} (C=O); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.08 (s, 1H, indole NH), 8.10 (t, *J* = 5.7 Hz, 1H, amide NH), 7.94–7.90

(m, 1H, amide NH), 7.75 (d, J = 2.0 Hz, 1H. ArH), 7.31 (dd, J = 8.6, 0.5 Hz, 1H, ArH), 7.27 (d, J = 2.4 Hz, 1H, ArH), 7.16 (dd, J = 8.6, 1.9 Hz, 1H, ArH), 3.67–3.63 (m, 2H, –CH_2–), 3.55 (br s, 2H, –CH_2–), 3.22–3.11 (m, 4H, –CH_2–), 2.80–2.74 (m, 3H, –CH_3), 1.38 (s, 9H, –CH_3); ^{13}C NMR (126 MHz, DMSO) δ ^{13}C NMR (126 MHz, DMSO) δ 28.1 (3C), 34.3 (br) (CH_3), 32.2, 37.0 (br), 42.1, 47.3 (br) (CH_2), 113.3, 121.2, 123.3, 125.6 (CH), 78.4 (br), 108.7, 111.0, 129.1, 134.8, 154.7 (br), 168.9, 170.7 (C); CHN: calc. C 51.40, H 5.82, N 11.99, found C 51.69, H 5.78, N 12.12; C20H27BrN4O4 (467.36); MS (ESI): m/z (%): pos.: 489.2 $[M+Na]^+$ (100), 955.2 $[2\,M+Na]^+$ (12), neg.: 465.2 $[M-H]^-$ (100); HPLC (isocr.): 98.5% at 254 nm, 99.3% at 280 nm, t_{M+S} = 5.24 min, t_M(DMSO) = 1.11 min (MeCN/water = 40:60); HPLC (grad.): 98.8% at 254 nm, t_{M+S} = 9.66 min, t_M(DMSO) = 1.15 min; λ_{max}: 226 nm, 288 nm; HR-MS (ESI): m/z $[M+Na]^+$ calc. 489.11079, found 489.11061.

***tert*-Butyl-*N*-(4-{2-[2-(5-bromo-1*H*-indole-3-yl)acetamido]-acetamido}butyl)-*N*-{3-[(*tert*-butoxycarbonyl)amino]propyl} carbamate (4d)**. According to General Procedure A from *N*-[2-(5-bromo-1*H*-indole-3-yl)acetyl]glycine (**3a**, 166 mg, 0.532 mmol), EDC·HCl (136 mg, 0.712 mmol), DIPEA (0.12 mL, 0.69 mmol) and *tert*-butyl-*N*-(4-aminobutyl)-*N*-{3-[(2-methylpropane-2-yl)-oxycarbonylamino]propyl}carbamate (176 mg, 0.510 mmol) in acetonitrile (2 mL) for 50.5 hours. Solvent for column chromatography: ethyl acetate/acetone (3:1). A colorless solid was obtained (270 mg, 0.422 mmol, 83%). M.p.: 67–70 °C; IR (KBr): 3302 cm^{-1} (N–H, br), 1666 cm^{-1} (C=O, br); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.08 (s, 1H, indole NH), 8.10 (t, J = 5.7 Hz, 1H, amide NH), 7.79–7.73 (m, 2H, amide NH, ArH), 7.31 (d, J = 8.6 Hz, 1H, ArH), 7.27 (d, J = 2.4 Hz, 1H, ArH), 7.16 (dd, J = 8.6, 2.0 Hz, 1H, ArH), 6.74 (m, 1H, carbamate NH), 3.65 (d, J = 5.7 Hz, 2H, –CH_2–), 3.55 (s, 2H, –CH_2–), 3.14–2.99 (m, 6H, –CH_2–), 2.88 (q, J = 6.5 Hz, 2H, –CH_2–), 1.57–1.53 (m, 2H, –CH_2–), 1.42–1.39 (m, 2H, –CH_2–), 1.39–1.32 (m, 18H, –CH_3), 1.34–1.26 (m, 2H, –CH_2–); ^{13}C NMR (126 MHz, DMSO) δ 28.1 (3 C), 28.2 (3 C) (CH_3), 25.2 (br), 26.4, 28.5 (br), 32.2, 37.6, 38.2, 42.1, 44.3 (br), 46.1 (br) (CH_2), 113.3, 121.1, 123.3, 125.6 (CH), 77.4, 78.3, 108.7, 111.0, 129.1, 134.8, 154.6, 155.5, 168.6, 170.8 (C); $C_{29}H_{44}BrN_5O_6$ (638.60); MS (ESI): m/z (%): pos.: 659.8 $[M+Na]^+$ (100), 675.8 $[M+K]^+$ (15), neg.: 561.8 $[M-76]^-$ (100), 635.9 $[M-H]^-$ (80); HPLC (isocr.): 96.6% at 254 nm, 99.4% at 280 nm, t_{M+S} = 6.29 min, t_M(DMSO) = 1.07 min (MeCN/water = 50:50); HPLC (grad.): 97.6% at 254 nm, t_{M+S} = 11.07 min, t_M(DMSO) = 1.14 min; λ_{max}: 226 nm, 289 nm; HR-MS (ESI): m/z $[M+H]^+$ calc. 638.25477, found 638.25551, $[M+Na]^+$ calc. 660.23672, found 660.23734.

***N*-(2-Aminoethyl)-2-[2-(5-bromo-1*H*-indole-3-yl)acetamido)-acetamide (4h)**. *tert*-Butyl-*N*-{*N*-[*N*-(5-bromo-1*H*-indole-3-ylacetyl)-glycyl]aminoethyl}carbamate (**4a**, 177 mg, 0.390 mmol) was suspended in dichloromethane (10 mL) and trifluoracetic acid (2.5 mL, 32 mmol) was added. The mixture was stirred for 4 hours at room temperature. The volatile components were evaporated under reduced pressure. The residue was taken up in water (10 mL), alkalized to pH = 10–11 with dilute aqueous sodium hydroxide and extracted with ethyl acetate (7×10 mL). The combined organic layers were dried over anhydrous sodium sulfate and the solvent evaporated under reduced pressure. A brown oil was obtained (31 mg, 0.09 mmol, 22%). IR (KBr): 3395 cm^{-1} (N–H, br), 1673 cm^{-1} (C=O, br); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.10 (s, 1H, indole NH), 8.14 (t, J = 5.6 Hz, 1H, amide NH), 7.87 (t, J = 5.8 Hz, 1H, amide NH), 7.75 (d, J = 2.0 Hz, 1H, ArH), 7.35–7.25 (m, 2H, ArH), 7.17 (dd, J = 8.6, 1.9 Hz, 1H, ArH), 3.68 (d, J = 5.7 Hz, 2H, –CH_2–), 3.59–3.53 (m, 2H, –CH_2–), 3.13 (q, J = 6.3 Hz, 2H, –CH_2–), 2.64 (t, J = 6.4 Hz, 2H, –CH_2–) (–NH_2 signal obscured); ^{13}C NMR (101 MHz, DMSO) δ 32.2, 40.3, 40.4, 42.2 (CH_2), 113.3, 121.1, 123.3, 125.5 (CH), 108.6, 111.0, 129.1, 134.7, 169.1, 170.8 (C); $C_{14}H_{17}BrN_4O_2$ (353.22); HPLC (isocr.): 85.3% at 254 nm, 93.2% at 280 nm, t_{M+S} = 6.63 min, t_M(DMSO) = 1.12 min (MeCN/buffer = 15:85); λ_{max}: 225 nm, 288 nm; HR-MS (ESI): m/z $[M+H]^+$ calc. 353.06076, found 353.06127.

***N*-(4-Aminobutyl)-2-[2-(5-bromo-1*H*-indole-3-yl)acetamido]-acetamide hydrochloride (4i)**. According to General Procedure B from *tert*-butyl-*N*-(4-{2-[2-(5-bromo-1*H*-indole-3-yl)acetamido]-acetamido}butyl)carbamate (**4b**, 55 mg, 0.11 mmol) and 3 M HCl in CPME (1.0 mL, 3.0 mmol) in dichloromethane (3 mL) for 24 hours. A sticky orange oil was obtained (51 mg, 0.12 mmol, quant.). IR (KBr): 3400 cm^{-1} (N–H, br), 2800–2400 cm^{-1} (N–H_3^+), 1648 cm^{-1} (C=O, br); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.13 (s, 1H, indole NH), 8.16 (t, J = 5.8 Hz, 1H, amide NH), 7.90–7.81 (m, 4H, amide NH, –NH_3^+), 7.75 (d, J = 1.9 Hz, 1H, ArH), 7.31 (d, J = 8.6 Hz, 1H, ArH), 7.28 (d, J = 2.4 Hz, 1H, ArH), 7.16 (dd, J = 8.6, 2.0 Hz, 1H, ArH), 3.66 (d, J = 5.7 Hz, 2H, –CH_2–), 3.56 (s, 2H, –CH_2–), 3.06 (q, J = 6.6 Hz, 2H, –CH_2–), 2.79–2.72 (m, 2H, –CH_2–), 1.56–1.48 (m, 2H, –CH_2–), 1.48–1.37 (m, 2H, –CH_2–); ^{13}C NMR (126 MHz, DMSO) δ 24.4, 26.0, 32.2, 37.8, 38.4, 42.2 (CH_2), 113.3, 121.2, 123.3, 125.6 (CH), 108.7, 111.0, 129.1, 134.8, 168.8, 170.8 (C); $C_{16}H_{22}BrClN_4O_2$ (417.73); MS (ESI): m/z (%): pos.: 380.9 $[M+H]^+$ (100), neg.: 378.9 $[M-H]^-$ (100); HPLC (isocr.): 91.2% at 254 nm, 98.0% at 280 nm, t_{M+S} = 7.05 min, t_M(DMSO) = 1.21 min (MeCN/buffer = 15:85); λ_{max}: 225 nm, 309 nm; HR-MS (ESI): m/z $[M+H]^+$ calc. 381.09206, found 381.09258.

2-(5-Bromo-1*H*-indole-3-yl)-*N*-(2-{[2-(methylamino)ethyl]amino}-2-oxoethyl)acetamide hydrochloride (4j). According to General Procedure B from *tert*-butyl-*N*-(2-{2-[2-(5-bromo-1*H*-indole-3-yl)-acetamido]acetamido}ethyl)(methyl)carbamate (**4c**, 52 mg, 0.11 mmol) and 3 M HCl in CPME (1.0 mL, 3.0 mmol) in dichloromethane (3 mL) for 24 hours. A sticky orange oil was obtained (43 mg, 0.12 mmol, quant.). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.12 (s, 1H, indole NH), 8.68 (br s, 2H, –NH_2^+–), 8.22–8.13 (m, 2H, amide NH), 7.76 (d, J = 2.0 Hz, 1H, ArH), 7.31 (d, J = 8.6 Hz, 1H, ArH), 7.28 (d, J = 2.4 Hz, 1H, ArH), 7.16 (dd, J = 8.6, 2.0 Hz, 1H, ArH), 3.71 (d, J = 5.7 Hz, 2H, –CH_2–), 3.58 (s, 2H, –CH_2–), 3.36 (q, J = 6.0 Hz, 2H, –CH_2–), 2.99–2.90 (m, 2H, –CH_2–), 2.53 (t, J = 5.5 Hz, 3H, –CH_3); ^{13}C NMR (126 MHz, DMSO) δ 32.4 (CH_3), 32.2, 34.9, 42.3, 47.7 (CH_2), 113.3, 121.2, 123.3, 125.6 (CH), 108.6, 111.0, 129.1, 134.8, 169.8, 170.9 (C); $C_{15}H_{20}BrClN_4O_2$ (403.71); MS (ESI): m/z (%): pos.: 366.8 $[M+H]^+$ (100), neg.: 364.9 $[M-H]^-$ (100); HPLC (isocr.): 95.5% at 254 nm, 96.1% at 280 nm, t_{M+S} = 6.79 min, t_M(DMSO) = 1.22 min (MeCN/buffer = 15:85); λ_{max}: 225 nm, 288 nm; HR-MS (ESI): m/z $[M+H]^+$ calc. 367.07641, found 367.07674.

***N*-{4-[(3-Aminopropyl)amino]butyl}-2-[2-(5-bromo-1*H*-indole-3-yl)-acetamido]acetamide dihydrochloride (4k)**. According to General Procedure B from *tert*-butyl-*N*-(4-{2-[2-(5-bromo-1*H*-indole-3-yl)-acetamido]acetamido}butyl)-*N*-{3-[(*tert*-butoxycarbonyl)amino]-propyl}carbamate (**4d**, 175 mg, 0.273 mmol) and 3M HCl in CPME (2 mL, 6 mmol) in 1,4-dioxane (6 mL) for 24 hours. A sticky orange oil was obtained (146 mg, 0.284 mmol, quant.). IR (KBr): 3399 cm^{-1} (N–H, br), 2800–2200 cm^{-1} (N-H_2^+/N-H_3^+), 1648 cm^{-1} (C=O, br); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.16 (s, 1H, indole NH), 9.05 (br s, 2H, –NH_2^+–), 8.20 (t, J = 5.8 Hz, 1H, amide NH), 8.12 (br s, 3H, –NH_3^+), 7.91 (t, J = 5.7 Hz, 1H, amide NH), 7.76 (d, J = 1.9 Hz, 1H, ArH), 7.32 (d, J = 8.6 Hz, 1H, ArH), 7.29 (d, J = 2.4 Hz, 1H, ArH), 7.16 (dd, J = 8.6, 1.9 Hz, 1H, ArH), 3.66 (d, J = 5.7 Hz, 2H, –CH_2–), 3.57 (s, 2H, –CH_2–), 3.07 (q, J = 6.6 Hz, 2H, –CH_2–), 2.99–2.79 (m, 6H, –CH_2–), 2.01–1.91 (m, 2H, –CH_2–), 1.66–1.56 (m, 2H, –CH_2–), 1.53–1.39 (m, 2H, –CH_2–); ^{13}C NMR (126 MHz, DMSO) δ 22.7, 23.6, 26.1, 32.2, 36.1, 37.8, 42.2, 43.7, 46.4 (CH_2), 113.3, 121.2, 123.3, 125.6 (CH), 108.7, 111.0, 129.1, 134.8, 168.8, 170.9 (C); $C_{19}H_{30}BrCl_2N_5O_2$ (511.29); MS (ESI): m/z (%): pos.: 437.8 $[M+H]^+$ (100), neg.: 435.9 $[M-H]^-$ (100); HPLC (isocr.): 81.5% at 254 nm, 95.1% at 280 nm, t_{M+S} = 5.07 min, t_M(DMSO) = 1.22 min (MeCN/buffer = 15:85); λ_{max}: 225 nm, 288 nm; HR-MS (ESI): m/z $[M+H]^+$ calc. 438.14991, found 438.15062.

2-(5-Bromo-1*H*-indole-3-yl)-*N*-{2-[*N*-benzylamino]-2-oxoethyl} acetamide (5a). According to General Procedure A from *N*-[2-(5-bromo-1*H*-indole-3-yl)acetyl]glycine (**3a**, 83 mg, 0.27 mmol), EDC·HCl (72 mg, 0.37 mmol), HOBt (52 mg, 0.39 mmol), DIPEA

(0.07 mL, 0.40 mmol) and benzylamine (0.05 mL, 0.59 mmol) in *N*,*N*-dimethylformamide (3 mL) for 30 hours. Solvent for column chromatography: dichloromethane/ethanol (20:1). A pale yellow solid was obtained (41 mg, 0.10 mmol, 39%). M.p.: 166–169 °C; IR (KBr): 3392 cm^{-1} (N–H), 3293 cm^{-1} (N–H, br), 1670 cm^{-1} (C=O), 1643 cm^{-1} (C=O); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.09 (s, 1H, indole NH), 8.35 (t, J = 6.0 Hz, 1H, amide NH), 8.19 (t, J = 5.8 Hz, 1H, amide NH), 7.76 (d, J = 2.0 Hz, 1H, ArH), 7.34–7.26 (m, 4H, ArH), 7.29–7.19 (m, 3H, ArH), 7.17 (dd, J = 8.5, 1.9 Hz, 1H, ArH), 4.28 (d, J = 6.0 Hz, 2H, $-CH_2-$), 3.74 (d, J = 5.8 Hz, 2H, $-CH_2-$), 3.56 (s, 2H, $-CH_2-$); ^{13}C NMR (126 MHz, DMSO) δ 32.2, 42.0, 42.2 (CH_2), 113.3, 121.2, 123.4, 125.6, 126.8, 127.2 (2 C), 128.3 (2 C) (CH), 108.7, 111.1, 129.2, 134.8, 139.3, 169.0, 170.9 (C); CHN: calc. C 57.01, H 4.53, N 10.50, found C 57.23, H 4.53, N 10.09; $C_{19}H_{18}BrN_3O_2$ (400.28); MS (ESI): m/z (%): pos.: 422.3 $[M+Na]^+$, neg.: 398.3 $[M-H]^-$ (100); HPLC (isocr.) 95.7% at 254 nm, 99.3 % at 280 nm, t_{M+S} = 4.45 min, t_M(DMSO) = 1.09 min (MeCN/water = 40:60); HPLC (grad.): 96.1% at 254 nm, t_{M+S} = 9.60 min, tM(DMSO) = 1.20 min; λ_{max}: 223 nm, 289 nm; HR-MS (ESI): m/z $[M+H]^+$ calc. 400.06552, found 400.06593, $[M+Na]^+$ calc. 422.04746, found 422.04806.

2-(5-Bromo-1*H*-indole-3-yl)-*N*-{2-[*N*-(4-chlorobenzyl)amino]-2-oxoethyl}acetamide (5 b). According to General Procedure A from *N*-[2-(5-bromo-1*H*-indole-3-yl)acetyl]glycine (**3 a**, 121 mg, 0.390 mmol), EDC·HCl (103 mg, 0.538 mmol), HOBt (75 mg, 0.56 mmol), DIPEA (0.10 mL, 0.56 mmol) and 4-chlorobenzylamine (0.07 mL, 0.57 mmol) in *N*,*N*-dimethylformamide (4 mL) for 46 hours. Solvent for column chromatography: dichloromethane→dichloromethane/ethanol (10:1). An off-white solid was obtained (37 mg, 0.09 mmol, 22%). M.p.: 183–184 °C; IR (KBr): 3292 cm^{-1} (N–H, br), 1627 cm^{-1} (C=O); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.09 (s, 1H, indole NH), 8.38 (t, J = 6.1 Hz, 1H, amide NH), 8.20 (t, J = 5.9 Hz, 1H, amide NH), 7.76 (d, J = 1.8 Hz, 1H, ArH), 7.37–7.33 (m, 2H, ArH), 7.31 (d, J = 8.7 Hz, 1H, ArH), 7.28 (d, J = 2.4 Hz, 1H, ArH), 7.27–7.23 (m, 2H, ArH), 7.17 (dd, J = 8.5, 2.0 Hz, 1H, ArH), 4.26 (d, J = 6.0 Hz, 2H, $-CH_2-$), 3.73 (d, J = 5.8 Hz, 2H, $-CH_2-$), 3.56 (s, 2H, $-CH_2-$); ^{13}C NMR (126 MHz, DMSO) δ 32.2, 41.3, 42.3 (CH_2), 113.3, 121.2, 123.4, 125.6, 128.2 (2C), 129.0 (2C) (CH), 108.7, 111.1, 129.2, 131.3, 134.8, 138.5, 169.1, 171.0 (C); $C_{19}H_{17}BrClN_3O_2$ (434.72); MS (ESI): m/z (%): pos.: 455.8 $[M+Na]^+$ (100), neg.: 431.8 $[M-H]^-$ (100); HPLC (isocr.) 96.5% at 254 nm, 98.3% at 280 nm, t_{M+S} = 3.25 min, t_M(DMSO) = 1.12 min (MeCN/water = 50:50); HPLC (grad.): 96.9% at 254 nm, t_{M+S} = 10.14 min, t_M(DMSO) = 1.16 min; λ_{max}: 223 nm, 289 nm; HR-MS (ESI): m/z $[M+Na]^+$ calc. 456.00849, found 456.00944.

2-(5-Bromo-1*H*-indole-3-yl)-*N*-{2-[*N*-(4-methoxybenzyl)amino]-2-oxoethyl}acetamide (5 c). According to General Procedure A from *N*-[2-(5-bromo-1*H*-indole-3-yl)acetyl]glycine (**3 a**, 271 mg, 0.872 mmol), EDC·HCl (236 mg, 1.23 mmol), HOBt (172 mg, 1.27 mmol), DIPEA (0.22 mL, 1.3 mmol) and 4-methoxybenzylamine (0.18 mL, 1.2 mmol) in *N*, *N*-dimethylformamide (9 mL) for 22 hours. Deviating from General Procedure A, water (20 mL) was added after the reaction and the mixture stored at 4 °C overnight. The precipitate was collected and washed with water. The solid (146 mg, 0.341 mmol) was recrystallized from ethanol/water (1:1,9 mL). A colorless solid was obtained (112 mg, 0.261 mmol, 77%). M.p.: 158–161 °C; IR (KBr): 3412 cm^{-1} (N–H), 3301 cm^{-1} (N–H), 3258 cm^{-1} (N–H), 1669 cm^{-1} (C=O), 1630 cm^{-1} (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.10 (s, 1H, indole NH), 8.28 (t, J = 6.0 Hz, 1H, amide NH), 8.19 (t, J = 5.8 Hz, 1H, amide NH), 7.76 (d, J = 2.0 Hz, 1H, ArH), 7.31 (d, J = 8.8 Hz, 1H, ArH), 7.28 (d, J = 2.5 Hz, 1H, ArH), 7.21–7.11 (m, 3H, ArH), 6.89–6.81 (m, 2H, ArH), 4.20 (d, J = 5.8 Hz, 2H, $-CH_2-$), 3.74–3.68 (m, 5H, $-CH_2-$, $-CH_3$), 3.56 (s, 2H, $-CH_2-$); ^{13}C NMR (101 MHz, DMSO) δ 55.1 (CH_3), 32.2, 41.5, 42.2 (CH_2), 113.3, 113.6 (2C), 121.2, 123.4, 125.6, 128.5 (2C) (CH), 108.7, 111.1, 129.2, 131.2, 134.8, 158.2, 168.8, 170.9 (C); CHN: calc. C 55.83, H 4.69, N 9.77, found C 55.78, H 4.73, N 9.38; $C_{20}H_{20}BrN_3O_3$ (430.30); MS (ESI): m/z (%): pos.: 452.4 $[M+Na]^+$ (100), neg.: 428.3 $[M-H]^-$ (100); HPLC (isocr.) 99.1% at 254 nm, 99.8% at 280 nm, t_{M+S} = 4.48 min, t_M(DMSO) = 1.10 min (MeCN/water = 40:60); HPLC (grad.): 96.3% at 254 nm, t_{M+S} = 9.56 min, t_M(DMSO) = 1.17 min; λ_{max}: 225 nm, 281 nm; HR-MS (ESI): m/z $[M+Na]^+$ calc. 452.05803, found 452.05875.

2-(5-Bromo-1*H*-indole-3-yl)-*N*-{2-[*N*-(4-methylbenzyl)amino]-2-oxoethyl}acetamide (5 d). According to General Procedure A from *N*-[2-(5-bromo-1*H*-indole-3-yl)acetyl]glycine (**3 a**, 135 mg, 0.434 mmol), EDC·HCl (116 mg, 0.604 mmol), DIPEA (0.11 mL, 0.63 mmol) and 4-methylbenzylamine (0.08 mL, 0.66 mmol) in acetonitrile (2 mL) for 21 hours. Solvent for column chromatography: toluene/ethanol (20:1)→toluene/ethanol (10:1). A colorless solid was obtained (39 mg, 0.09 mmol, 22%). M.p.: 169–171 °C; IR (KBr): 3258 cm^{-1} (N–H, br), 1668 cm^{-1} (C=O), 1631 cm^{-1} (C=O); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.09 (s, 1H, indole NH), 8.29 (t, J = 6.0 Hz, 1H, amide NH), 8.18 (t, J = 5.8 Hz, 1H, amide NH), 7.76 (d, J = 2.0 Hz, 1H, ArH), 7.31 (d, J = 8.5 Hz, 1H, ArH), 7.28 (d, J = 2.4 Hz, 1H, ArH), 7.17 (dd, J = 8.6, 2.0 Hz, 1H, ArH), 7.17–7.07 (m, 4H, ArH), 4.23 (d, J = 6.0 Hz, 2H, $-CH_2-$), 3.72 (d, J = 5.8 Hz, 2H, $-CH_2-$), 3.56 (s, 2H, $-CH_2-$), 2.26 (s, 3H, $-CH_3$); ^{13}C NMR (126 MHz, DMSO) δ 20.7 (CH_3), 32.2, 41.8, 42.2 (CH_2), 113.3, 121.2, 123.4, 125.6, 127.2 (2 C), 128.8 (2C) (CH), 108.7, 111.1, 129.2, 134.8, 135.8, 136.3, 168.9, 170.9 (C); CHN: calc. C 57.98, H 4.87, N 10.14, found C 57.99, H 4.82, N 9.85; $C_{20}H_{20}BrN_3O_2$ (414.30); MS (ESI): m/z (%): pos.: 435.9 $[M+Na]^+$ (100), neg.: 411.8 $[M-H]^-$ (100); HPLC (isocr.) 98.7% at 254 nm, 99.4% at 280 nm, t_{M+S} = 3.07 min, t_M(DMSO) = 1.12 min (MeCN/water = 50:50); HPLC (grad.): 97.8% at 254 nm, t_{M+S} = 10.00 min, t_M(DMSO) = 1.17 min; λ_{max}: 221 nm, 289 nm; HR-MS (ESI): m/z $[M+H]^+$ calc. 414.08117, found:414.08159, $[M+Na]^+$ calc. 436.06311, found 436.06364.

2-(5-Bromo-1*H*-indole-3-yl)-*N*-{2-[*N*-(3,4-dichlorobenzyl)amino]-2-oxoethyl}acetamide (5 e). According to General Procedure A from *N*-[2-(5-bromo-1*H*-indole-3-yl)acetyl]glycine (**3 a**, 130 mg, 0.419 mmol), HOBt (81 mg, 0.60 mmol), EDC·HCl (115 mg, 0.600 mmol), DIPEA (0.11 mL, 0.63 mmol) and 3,4-dichlorobenzylamine (0.08 mL, 0.60 mmol) in *N*,*N*-dimethylformamide (4 mL) for 33.5 hours. Solvent for column chromatography: toluene/dichloromethane/ethanol (10:4:1). An off-white solid was obtained (41 mg, 0.09 mmol, 21%). M.p.: 172–174 °C; IR (KBr): 3461 cm^{-1} (N–H), 3248 cm^{-1} (N–H, br), 1677 cm^{-1} (C=O), 1636 cm^{-1} (C=O); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.09 (s, 1H, indole NH), 8.42 (t, J = 6.1 Hz, 1H, amide NH), 8.23 (t, J = 5.9 Hz, 1H, amide NH), 7.76 (d, J = 2.0 Hz, 1H, ArH), 7.55 (d, J = 8.2 Hz, 1H, ArH), 7.50 (d, J = 2.1 Hz, 1H, ArH), 7.31 (d, J = 8.7 Hz, 1H, ArH), 7.28 (d, J = 2.4 Hz, 1H, ArH), 7.23 (dd, J = 8.2, 2.1 Hz, 1H, ArH), 7.16 (dd, J = 8.6, 2.0 Hz, 1H, ArH), 4.28 (d, J = 5.9 Hz, 2H, $-CH_2-$), 3.73 (d, J = 5.7 Hz, 2H, $-CH_2-$), 3.56 (s, 2H, $-CH_2-$);^{13}C NMR (126 MHz, DMSO) δ 32.2, 40.9, 42.3 (CH_2), 113.3, 121.2, 123.4, 125.6, 127.5, 129.1, 130.4 (CH), 108.6, 111.1, 129.2, 129.2, 130.9, 134.8, 140.8, 169.3, 171.0 (C); $C_{19}H_{16}BrCl_2N_3O_2$ (469.16); MS (ESI):m/z (%): pos.: 489.7 $[M+Na]^+$ (100), neg.: 465.7 $[M-H]^-$ (100); HPLC (isocr.) 95.3% at 254 nm, 99.5% at 280 nm, t_{M+S} = 4.24 min, t_M(DMSO) = 1.12 min (MeCN/water = 50:50); HPLC (grad.): 98.1% at 254 nm, t_{M+S} = 10.57 min, t_M(DMSO) = 1.16 min; λ_{max}: 224 nm, 280 nm, 288 nm; HR-MS (ESI):m/z $[M+Na]^+$ calc. 489.96952, found 489.96940.

2-(5-Bromo-1*H*-indole-3-yl)-*N*-{2-[*N*-(4-hydroxybenzyl)amino]-2-oxoethyl}acetamide (5 f). 2-(5-bromo-1*H*-indole-3-yl)-*N*-{2-[*N*-(4-methoxybenzyl)amino]-2-oxoethyl}acetamide (**5 c**, 66 mg, 0.15 mmol) was suspended in dried dichloromethane (4 mL) and boron tribromide (1M in dichloromethane, 1.0 mL, 1.0 mmol) was added. The mixture was stirred at room temperature for 17.5 hours, after which TLC indicated complete conversion of the ether. Water (10 mL) was added, and the biphasic mixture stirred for 2 hours at room temperature. The solid was collected by filtration and washed

with dichloromethane and water. A pale brown solid was obtained (47 mg, 0.11 mmol, 74%). M.p.: 194–196 °C; IR (KBr): 3328 cm^{-1} (N–H), 3247 cm^{-1} (N–H, br), 1655 cm^{-1} (C=O), 1630 cm^{-1} (C=O), 1245 cm^{-1} (O–CH_3); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.09 (s, 1H, indole NH), 9.26 (s, 1H, –OH), 8.22 (t, J = 5.9 Hz, 1H, amide NH), 8.15 (t, J = 5.8 Hz, 1H, amide NH), 7.76 (d, J = 1.8 Hz, 1H, Ar), 7.31 (d, J = 8.5 Hz, 1H, ArH), 7.28 (d, J = 2.4 Hz, 1H, ArH), 7.17 (dd, J = 8.5, 2.0 Hz, 1H, ArH), 7.07–7.00 (m, 2H, ArH), 6.71–6.65 (m, 2H, ArH), 4.15 (d, J = 5.9 Hz, 2H, –CH_2–), 3.70 (d, J = 5.8 Hz, 2H, –CH_2–), 3.55 (s, 2H, –CH_2–); ^{13}C NMR (126 MHz, DMSO) δ 32.2, 41.6, 42.2 (CH_2), 113.3, 115.0 (2C), 121.2, 123.4, 125.6, 128.6 (2C) (CH_2), 108.7, 111.1, 129.2, 129.4, 134.8, 156.2, 168.7, 170.8 (C); $C_{19}H_{18}BrN_3O_3$ (416.28); MS (ESI): m/z (%): pos.: 437.9 $[M+Na]^+$ (100), neg.: 413.8 $[M-H]^-$(100), 307.8. $[M-C_7H_7O]^-$ (73); HPLC (isocr.) 98.7% at 254 nm, 99.6% at 280 nm, t_{M+S} = 4.97 min, t_M(DMSO) = 1.20 min (MeCN/water = 30:70); HPLC (grad.): 95.5% at 254 nm, t_{M+S} = 8.52 min, t_M(DMSO) = 1.15 min; λ_{max}: 225 nm, 281 nm; HR-MS (ESI): m/z $[M+H]^+$ calc. 416.06043, found 406.06096, $[M+Na]^+$ calc. 438.04238, found 438.04300.

2-(5-Bromo-1*H*-indole-3-yl)-*N*-{2-[*N*-(2-chlorobenzyl)amino]-2-oxoethyl}acetamide (5g). According to General Procedure A from *N*-[2-(5-bromo-1*H*-indole-3-yl)acetyl]glycine (**3a**, 93 mg, 0.30 mmol), HOBt (56 mg, 0.42 mmol), EDC·HCl (86 mg, 0.45 mmol), DIPEA (0.08 mL, 0.46 mmol) and 2-chlorobenzylamine (0.06 mL, 0.50 mmol) in *N*,*N*-dimethylformamide (3 mL) for 25 hours. Solvent for column chromatography: dichloromethane/ethanol (20:1). A pale brown solid was obtained (70 mg, 0.16 mmol, 54%). M.p.: 180–182 °C; IR (KBr): 3394 cm^{-1} (N–H), 3282 cm^{-1} (N–H, br), 1673 cm^{-1} (C=O), 1639 cm^{-1} (C=O); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.09 (s, 1H, indole NH), 8.38 (t, J = 5.9 Hz, 1H, amide NH), 8.23 (t, J = 5.8 Hz, 1H, amide NH), 7.76 (d, J = 1.9 Hz, 1H, ArH), 7.46–7.39 (m, 1H, ArH), 7.36–7.24 (m, 5H, ArH), 7.16 (dd, J = 8.7, 2.0 Hz, 1H, ArH), 4.34 (d, J = 6.0 Hz, 2H, –CH_2–), 3.77 (d, J = 5.7 Hz, 2H, –CH_2–), 3.57 (d, J = 0.9 Hz, 2H, –CH_2–); ^{13}C NMR (126 MHz, DMSO) δ 32.2, 39.9 (overlapping with the DMSO signal), 42.2 (CH_2), 113.3, 121.2, 123.4, 125.6, 127.1, 128.6, 128.7, 129.0 (CH), 108.7, 111.1, 129.2, 131.9, 134.8, 136.2, 169.3, 171.0 (C); $C_{19}H_{17}BrClN_3O_2$ (434.72); MS (ESI): m/z (%): pos.: 456.3 $[M+Na]^+$ (100), neg.: 432.3 $[M-H]^-$ (100); HPLC (isocr.) 98.4% at 254 nm, 99.9% at 280 nm, t_{M+S} = 6.21 min, t_M(DMSO) = 1.10 min (MeCN/water = 40:60); HPLC (grad.): 98.2% at 254 nm, t_{M+S} = 9.99 min, t_M(DMSO) = 1.19 min; λ_{max}: 222 nm, 289 nm; HR-MS (ESI): $[M+Na]^+$ calc. 456.00849, found 456.00928.

2-(5-Bromo-1*H*-indole-3-yl)-*N*-(2-oxo-2-{*N*-[4-(trifluoromethyl)-benzyl]amino}ethyl)acetamide (5h). According to General Procedure A from *N*-[2-(5-bromo-1*H*-indole-3-yl)acetyl]glycine (**3a**, 87 mg, 0.28 mmol), HOBt (54 mg, 0.40 mmol), EDC·HCl (77 mg, 0.40 mmol), DIPEA (0.07 mL, 0.40 mmol) and 4-(trifluoromethyl)-benzylamine (0.06 mL, 0.41 mmol) in *N*,*N*-dimethylformamide (3 mL) for 22 hours. Solvent for column chromatography: dichloromethane/ethanol (100:1)→dichloromethane/ethanol (10:1). A pale yellow solid was obtained (73 mg, 0.16 mmol, 56%). M.p.: 148–149 °C; IR (KBr): 3295 cm^{-1} (N–H, br), 1650 cm^{-1} (C=O, br); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.09 (s, 1H, indole NH), 8.46 (t, J = 6.1 Hz, 1H, amide NH), 8.23 (t, J = 5.8 Hz, 1H, amide NH), 7.76 (d, J = 1.9 Hz, 1H, ArH), 7.65 (d, J = 8.2 Hz, 2H, ArH), 7.45 (d, J = 8.0 Hz, 2H, ArH), 7.31 (d, J = 8.5 Hz, 1H, ArH), 7.28 (d, J = 2.4 Hz, 1H, ArH), 7.16 (dd, J = 8.6, 2.0 Hz, 1H, ArH), 4.37 (d, J = 5.9 Hz, 2H, –CH_2–), 3.75 (d, J = 5.8 Hz, 2H, –CH_2–), 3.57 (s, 2H, –CH_2–); ^{13}C NMR (126 MHz, DMSO) δ 32.1, 41.5, 42.2 (CH_2), 113.2, 121.1, 123.3, 125.0 (q, J = 3.7 Hz), 125.5, 127.6 (2C) (CH), 108.6, 110.9, 124.3 (q, J = 272.9 Hz), 127.3 (q, J = 31.5 Hz), 129.0, 134.7, 144.2, 169.1, 170.9 (C); $C_{20}H_{17}BrF_3N_3O_2$ (468.27); MS (ESI): m/z (%): pos.: 490.4 $[M+Na]^+$ (100), neg.: 466.4 $[M-H]^-$ (100); HPLC (isocr.) 98.1% at 254 nm, 99.7% at 280 nm, t_{M+S} = 9.05 min, t_M(DMSO) = 1.08 min (MeCN/water = 40:60); HPLC (grad.): 96.0% at 254 nm, t_{M+S} = 10.36 min, t_M(DMSO) = 1.19 min; λ_{max}: 222 nm, 289 nm; HR-MS (ESI): m/z $[M+H]^+$ calc. 468.05290, found 468.05276, $[M+Na]^+$ calc. 490.03485, found 490.03466.

2-(5-Bromo-1*H*-indole-3-yl)-*N*-(2-{[4-chloro-3-(trifluoromethyl)-benzyl]amino}-2-oxoethyl)acetamide (5i). According to General Procedure A from *N*-[2-(5-bromo-1*H*-indole-3-yl)acetyl]glycine (**3a**, 120 mg, 0.385 mmol), EDC·HCl (104 mg, 0.540 mmol), DIPEA (0.15 mL, 0.86 mmol) and 4-chloro-3-(trifluoromethyl)benzylamine (0.19 mL, 1.3 mmol) in acetonitrile (3 mL) for 72 hours. Solvent for column chromatography: Ethyl acetate. A colorless solid was obtained (108 mg, 0.216 mmol, 56%). M.p.: 162–163 °C; IR (KBr): 3417 cm^{-1} (N–H, br), 3282 cm^{-1} (N–H, br), 1631 cm^{-1} (C=O, br); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.09 (s, 1H, indole NH), 8.47 (t, J = 6.1 Hz, 1H, amide NH), 8.23 (t, J = 5.8 Hz, 1H, amide NH), 7.77–7.72 (m, 2H, ArH), 7.66–7.62 (m, 1H, ArH), 7.54 (dd, J = 8.4, 2.2 Hz, 1H, ArH), 7.31 (dd, J = 8.6, 0.6 Hz, 1H, ArH), 7.28 (d, J = 2.4 Hz, 1H, ArH), 7.16 (dd, J = 8.6, 2.0 Hz, 1H, ArH), 4.35 (d, J = 6.0 Hz, 2H, –CH_2–), 3.73 (d, J = 5.8 Hz, 2H, –CH_2–), 3.56 (s, 2H, –CH_2–); ^{13}C NMR (101 MHz, DMSO) δ 32.2, 41.1, 42.3 (CH_2), 113.3, 121.1, 123.3, 125.5, 126.5 (q, J = 5.2 Hz, 131.4, 132.8, (CH), 108.6, 111.0, 128.8 (q, J = 1.8 Hz), 129.1, 134.7, 139.7, 169.3, 170.9 (C) (two carbon signals are missing: –**C**F_3 and –**C**–CF_3); $C_{20}H_{16}BrClF_3N_3O_2$ (502.72); MS (ESI): m/z (%): pos.: 524.1 $[M+Na]^+$ (100), neg.: 500.0 $[M-H]^-$ (100); HPLC (isocr.): 92.7% at 254 nm, 96.2% at 280 nm, t_{M+S} = 4.97 min, t_M(DMSO) = 1.15 min (MeCN/water = 50:50); HPLC (grad.): 98.0% at 254 nm, t_{M+S} = 10.77 min, t_M(DMSO) = 1.16 min; λ_{max}: 223 nm, 280 nm; HR-MS (ESI): m/z $[M+Na]^+$ calc. 523.99587, found 523.99613.

***tert*-Butyl-*N*-(4-{2-[2-(5-bromo-2-phenyl-1*H*-indole-3-yl)-acetamido]acetamido}butyl)carbamate (6a)**. According to General Procedure A from *N*-[2-(5-Bromo-2-phenyl-1*H*-indole-3-yl)acetyl]-glycine (**3d**, 204 mg, 0.528 mmol), EDC·HCl (147 mg, 0.767 mmol), DIPEA (0.22 mL, 1.2 mmol) and *N*-(*tert*-butoxycarbonyl)-1,4-diaminobutane (0.14 mL, 0.74 mmol) in dichloromethane (2 mL) for 68 hours. Solvent for column chromatography: dichloromethane/ethyl acetate (2:3)→ethyl acetate. A colorless solid was obtained (44 mg, 0.08 mmol, 15%). M.p.: 175–177 °C; IR (KBr): 3391 cm^{-1} (N–H), 3295 cm^{-1} (N–H), 1683 cm^{-1} (C=O), 1639 cm^{-1} (C=O); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.49 (s, 1H, indole NH), 8.26 (t, J = 5.8 Hz, 1H, amide NH), 7.82–7.78 (m, 3H, ArH), 7.76 (t, J = 5.6 Hz, 1H, amide NH), 7.55–7.47 (m, 2H, ArH), 7.45–7.38 (m, 1H, ArH), 7.33 (dd, J = 8.5, 0.5 Hz, 1H, ArH), 7.21 (dd, J = 8.5, 2.0 Hz, 1H, ArH), 6.75 (t, J = 5.6 Hz, 1H, carbamate NH), 3.70 (d, J = 5.7 Hz, 2H, –CH_2–), 3.66 (s, 2H, –CH_2–), 3.08–3.01 (m, 2H, –CH_2–), 2.93–2.86 (m, 2H, –CH_2–), 1.37 (m, 13H, –CH_2–, –CH_3); ^{13}C NMR (126 MHz, DMSO) δ 28.3 (3C) (CH_3), 26.5, 26.9, 31.4, 38.3, 39.5 (overlapping with the DMSO signal), 42.2 (CH_2), 113.0, 121.2, 124.0, 127.9, 128.2 (2C), 128.7 (2C) (CH), 77.3, 105.7, 111.4, 130.7, 131.9, 134.5, 137.4, 155.6, 168.5, 170.8 (C); $C_{27}H_{33}BrN_4O_4$ (557.49); MS (ESI): m/z (%): pos.: 579.2 $[M+Na]^+$ (100), neg.: 555.2 $[M-H]^-$ (100); HPLC (isocr.): 99.3% at 254 nm, 99.5% at 280 nm, t_{M+S} = 4.94 min, t_M(DMSO) = 1.12 min (MeCN/water = 50:50); HPLC (grad.): 98.3% at 254 nm, t_{M+S} = 10.74 min, t_M(DMSO) = 1.15 min; λ_{max}: 232 nm, 309 nm; HR-MS (ESI): m/z $[M+H]^+$ calc. 557.17579, found 557.17620, $[M+Na]^+$ calc. 579.15774, found 579.15830.

***tert*-Butyl-*N*-(2-{2-[2-(5-bromo-2-phenyl-1*H*-indole-3-yl)-acetamido]acetamido}ethyl)-*N*-(methyl)carbamate (6b)**. According to General Procedure A from *N*-[2-(5-bromo-2-phenyl-1*H*-indole-3-yl)acetyl]glycine (**3d**, 180 mg, 0.465 mmol), EDC·HCl (126 mg, 0.658 mmol), DIPEA (0.20 mL, 1.1 mmol) and *N*-(*tert*-butoxycarbonyl)-*N*-methylethylenediamine (0.12 mL, 0.65 mmol) in dichloromethane (1 mL) for 64 hours. Solvent for column chromatography: ethyl acetate. A colorless solid was obtained (211 mg, 0.388 mmol, 83%). M.p.: 147 °C (decomp.); IR (KBr): 3386 cm^{-1} (N–H, br), 3291 cm^{-1} (N–H, br), 1644 cm^{-1} (C=O, br); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.49 (s, 1H, indole NH), 8.28 (t, J = 5.7 Hz, 1H, amide NH), 7.96–7.89 (m, 1H, amide NH), 7.82–7.76 (m, 3H, ArH), 7.54–7.47

(m, 2H, ArH), 7.45–7.38 (m, 1H, ArH), 7.33 (d, J = 8.5 Hz, 1H, ArH), 7.21 (dd, J = 8.5, 2.0 Hz, 1H, ArH), 3.74–3.68 (m, 2H, $-CH_2-$), 3.67 (s, 2H, $-CH_2$), 3.21–3.17 (m, 4H, $-CH_2-$), 2.80–2.75 (m, 3H, $-CH_3$), 1.38 (s, 9H, $-CH_3$); ^{13}C NMR (126 MHz, DMSO) δ 28.1 (3C), 34.5 (br) (CH_3), 31.4, 36.9 (br), 42.2, 47.6 (br) (CH_2), 113.0, 121.2, 124.0, 127.9, 128.2 (2C), 128.7 (2C) (CH), 78.4, 105.7, 111.4, 130.7, 131.9, 134.5, 137.4, 154.8 (br), 168.9 (br), 170.9 (C); $C_{26}H_{31}BrN_4O_4$ (543.46); MS (ESI): m/z (%): pos.: 565.2 $[M+Na]^+$ (100), neg.: 541.2 $[M-H]^-$ (100); HPLC (isocr.): 98.7% at 254 nm, 97.9% at 280 nm, t_{M+S} = 5.45 min, t_M(DMSO) = 1.12 min (MeCN/water = 50:50); HPLC (grad.): 98.4% at 254 nm, t_{M+S} = 10.92 min, t_M(DMSO) = 1.27 min; λ_{max}: 232 nm, 309 nm; HR-MS (ESI): m/z $[M+Na]^+$ calc. 565.14209, found 565.14263.

***tert*-Butyl-*N*-(4-{2-[2-(5-bromo-2-phenyl-1H-indole-3-yl)-acetamido]acetamido}butyl)-*N*-{3-[(*tert*-butoxycarbonyl)amino]-propyl}carbamate (6c)**. According to General Procedure A from *N*-[2-(5-bromo-2-phenyl-1*H*-indole-3-yl)acetyl]glycine (**3d**, 100 mg, 0.258 mmol), EDC·HCl (70 mg, 0.37 mmol), DIPEA (0.07 mL, 0.40 mmol) and *tert*-butyl-N-(4-aminobutyl)-N-{3-[(2-methyl-propane-2-yl)oxycarbonylamino]propyl}carbamate (70 mg, 0.20 mmol) in acetonitrile (2 mL) for 50.5 hours. Solvent for column chromatography: ethyl acetate/petroleum ethers (16:3). A pale yellow solid was obtained (82 mg, 0.12 mmol, 57%). M.p.: 138–140 °C; IR (KBr): 3299 cm^{-1} (N–H, br), 1709 cm^{-1} (C=O), 1660 cm^{-1} (C=O), 1631 cm^{-1} (C=O); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.48 (s, 1H, indole NH), 8.27 (t, J = 5.8 Hz, 1H, amide NH), 7.81–7.74 (m, 4H, ArH, amide NH), 7.54–7.47 (m, 2H, ArH), 7.45–7.38 (m, 1H, ArH), 7.32 (d, J = 8.5 Hz, 1H, ArH), 7.21 (dd, J = 8.6, 1.9 Hz, 1H, ArH), 6.73 (br s, 1H, carbamate NH), 3.70 (d, J = 5.7 Hz, 2H, $-CH_2-$), 3.66 (s, 2H, $-CH_2-$), 3.11–3.03 (m, 6H, $-CH_2-$), 2.87 (q, J = 6.5 Hz, 2H, $-CH_2-$), 1.56–1.53 (m, 2H, $-CH_2-$), 1.37 (m, 22H, $-CH_2-$, $-CH_3$); ^{13}C NMR (126 MHz, DMSO) δ 28.1 (3C), 28.2 (3C) (CH_3), 26.5, 31.4, 37.6, 38.3, 42.2 (CH_2), 113.0, 121.2, 124.0, 127.9, 128.2 (2C), 128.7 (2C) (CH), 77.4, 78.3, 105.7, 111.4, 130.7, 131.9, 134.5, 137.4, 154.6, 155.5, 168.5, 170.9 (four $-CH_2-$ are poorly resolved); $C_{35}H_{48}BrN_5O_6$ (714.70); MS (ESI): m/z (%): pos.: 735.8 $[M+Na]^+$ (100), 751.8 $[M+K]^+$ (30), neg.: 711.9 $[M-H]^-$ (100); HPLC (isocr.): 98.3% at 254 nm, 99.1% at 280 nm, t_{M+S} = 5.40 min, t_M(DMSO) = 1.10 min (MeCN/water = 60:40); HPLC (grad.): 98.2% at 254 nm, t_{M+S} = 12.09 min, t_M(DMSO) = 1.15 min; λ_{max}: 232 nm, 309 nm; HR-MS (ESI): m/z $[M+Na]^+$ calc. 736.26802, found 736.26881.

***N*-(4-Aminobutyl)-2-[2-(5-bromo-2-phenyl-1*H*-indole-3-yl)-acetamido]acetamide hydrochloride (6d)**. According to General Procedure B from *tert*-butyl-*N*-(4-{2-[2-(5-bromo-2-phenyl-1*H*-indole-3-yl)acetamido]acetamido}butyl)carbamate (**6a**, 54 mg, 0.10 mmol) and 3 M HCl in CPME (1 mL, 3 mmol) in 1,4-dioxane (3 mL) for 24 hours. An orange solid was obtained (57 mg, 0.12 mmol, quant.). M.p.: 148 °C (decomp.); IR (KBr): 3401 cm^{-1} (N–H), 3259 cm^{-1} (N–H), 2800–2200 cm^{-1} (N–H_3^+), 1652 cm^{-1} (C=O, br); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.53 (s, 1H, indole NH), 8.33 (t, J = 5.8 Hz, 1H, amide NH), 7.89 (t, J = 5.8 Hz, 1H, amide NH), 7.85–7.76 (m, 6H, $-NH_3^+$, ArH), 7.51 (m, 2H, ArH), 7.45–7.38 (m, 1H, ArH), 7.33 (d, J = 8.5 Hz, 1H, ArH), 7.21 (dd, J = 8.6, 1.9 Hz, 1H, ArH), 3.71 (d, J = 5.7 Hz, 2H, $-CH_2-$), 3.67 (s, 2H, $-CH_2-$), 3.08 (q, J = 6.6 Hz, 2H, $-CH_2-$), 2.81–2.71 (m, 2H, $-CH_2-$), 1.58–1.48 (m, 2H, $-CH_2-$), 1.50–1.37 (m, 2H, $-CH_2-$); ^{13}C NMR (126 MHz, DMSO) δ 24.4, 26.1, 31.4, 37.8, 38.5, 42.2 (CH_2), 113.0, 121.2, 124.0, 127.9, 128.2 (2C), 128.7 (2C) (CH), 105.7, 111.4, 130.7, 131.9, 134.5, 137.4, 168.7, 170.9 (C); $C_{22}H_{26}BrClN_4O_2$ (493.83); MS (ESI): m/z (%): pos.: 456.8 $[M+H]^+$ (100), neg.: 454.9 $[M-H]^-$ (100); HPLC (isocr.): 98.5% at 254 nm, 98.6% at 280 nm, t_{M+S} = 3.21 min, t_M(DMSO) = 1.13 min (MeCN/buffer = 30:70); λ_{max}: 233 nm, 309 nm; HR-MS (ESI): m/z $[M+H]^+$ calc. 457.12337, found 487.12453.

2-(5-Bromo-2-phenyl-1*H*-indole-3-yl)-*N*-(2-{[2-(methylamino)-ethyl]amino}-2-oxoethyl)acetamide hydrochloride (6e). According to General Procedure B from *tert*-butyl-*N*-(2-{2-[2-(5-bromo-2-phenyl-1*H*-indole-3-yl)acetamido]acetamido}ethyl)-*N*-(methyl)-carbamate (**6b**, 60 mg, 0.11 mmol) and 3 M HCl in CPME (1 mL, 3 mmol) in 1,4-dioxane (3 mL) for 24 hours. An orange solid was obtained (66 mg, 0.14 mmol, quant.). M.p.: 115 °C (decomp.); IR (KBr): 3400 cm^{-1} (N–H), 3252 cm^{-1} (N–H, br), 2800–2000 cm^{-1} (N-H_2^+), 1644 cm^{-1} (C=O, br); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.53 (s, 1H, indole NH), 8.72 (br s, 2H, $-NH_2^+-$), 8.38 (t, J = 5.7 Hz, 1H, amide NH), 8.20 (t, J = 5.7 Hz, 1H, amide NH), 7.84–7.76 (m, 3H, ArH), 7.55–7.48 (m, 2H, ArH), 7.45–7.38 (m, 1H, ArH), 7.33 (d, J = 8.6 Hz, 1H, ArH), 7.21 (dd, J = 8.6, 2.0 Hz, 1H, ArH), 3.77 (d, J = 5.7 Hz, 2H, $-CH_2-$), 3.69 (s, 2H, $-CH_2-$), 3.38 (q, J = 6.1 Hz, 2H, $-CH_2-$), 2.98–2.94 (m, 2H, $-CH_2-$), 2.54 (br s, 3H, $-CH_3$); ^{13}C NMR (126 MHz, DMSO) δ 32.4 (CH_3), 31.4, 35.0, 42.4, 47.7 (CH_2), 113.0, 121.3, 124.0, 127.9, 128.2 (2C), 128.7 (2C) (CH), 105.6, 111.4, 130.7, 131.9, 134.5, 137.4, 169.7, 171.0 (C); $C_{21}H_{24}BrClN_4O_2$ (479.80); MS (ESI): m/z (%): pos.: 442.8 $[M+H]^+$ (100), neg.: 440.9 $[M-H]^-$ (100); HPLC (isocr.): 98.8% at 254 nm, 99.1% at 280 nm, t_{M+S} = 3.17 min, t_M(DMSO) = 1.13 min (MeCN/buffer = 30:70); λ_{max}: 232 nm, 309 nm; HR-MS (ESI): m/z $[M+H]^+$ calc. 443.10772, found 443.10859.

***N*-{4-[(3-Aminopropyl)amino]butyl}-2-[2-(5-bromo-2-phenyl-1*H*-indole-3-yl)acetamido]acetamide dihydrochloride (6f)**. According to General Procedure B from *tert*-butyl-*N*-(4-{2-[2-(5-bromo-2-phenyl-1*H*-indole-3-yl)acetamido]acetamido}butyl)-*N*-{3-[(*tert*-butoxycarbonyl)amino]propyl}carbamate (**6c**, 45 mg, 0.06 mmol) and 3 M HCl in CPME (1 mL, 3 mmol) in dichloromethane (3 mL) for 24 hours. An off-white solid was obtained (39 mg, 0.07 mmol, quant.). M.p.: 137 °C (decomp.); IR (KBr): 3403 cm^{-1} (N–H, br), 2800–2000 cm^{-1} (N-H_2^+/N-H_3^+), 1646 cm^{-1} (C=O, br); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.54 (s, 1H, indole NH), 8.98 (br s, 2H, $-NH_2^+-$), 8.35 (t, J = 5.8 Hz, 1H, amide NH), 8.07 (br s, 3H, $-NH_3^+$), 7.91 (t, J = 5.7 Hz, 1H, amide NH), 7.84–7.77 (m, 3H, ArH), 7.54–7.48 (m, 2H, ArH), 7.46–7.38 (m, 1H, ArH), 7.34 (d, J = 8.5 Hz, 1H, ArH), 7.21 (dd, J = 8.5, 1.9 Hz, 1H, ArH), 3.72 (d, J = 5.8 Hz, 2H, $-CH_2-$), 3.68 (s, 2H, $-CH_2-$), 3.09 (q, J = 6.6 Hz, 2H, $-CH_2-$), 2.99–2.80 (m, 6H, $-CH_2-$), 2.00–1.90 (m, 2H, $-CH_2-$), 1.67–1.57 (m, 2H, $-CH_2-$), 1.50–1.39 (m, 2H, $-CH_2-$); ^{13}C NMR (126 MHz, DMSO) δ 22.8, 23.6, 26.2, 31.4, 36.1, 37.8, 42.3, 43.7, 46.4 (CH_2), 113.0, 121.2, 124.0, 127.9, 128.2 (2C), 128.7 (2C) (CH), 105.7, 111.4, 130.7, 131.9, 134.5, 137.4, 168.7, 170.9 (C); $C_{25}H_{34}BrCl_2N_5O_2$ (587.38); MS (ESI): m/z (%): pos.: 513.9 $[M+H]^+$ (100), neg.: 511.9 $[M-H]^-$ (100); HPLC (isocr.): 98.3% at 254 nm, 99.4% at 280 nm, t_{M+S} = 2.25 min, t_M(DMSO) = 1.13 min (MeCN/buffer = 20:80); λ_{max}: 232 nm, 309 nm; HR-MS (ESI): m/z $[M+H]^+$ calc. 514.18121, found 514.18157.

2-(5-Bromo-2-phenyl-1*H*-indole-3-yl)-*N*-(2-{[4-chloro-3-(trifluoromethyl)benzyl]amino}-2-oxoethyl)acetamide (6g). According to General Procedure A from *N*-[2-(5-bromo-2-phenyl-1*H*-indole-3-yl)acetyl]glycine (**3d**, 76 mg, 0.20 mmol), EDC·HCl (58 mg, 0.30 mmol), DIPEA (0.09 mL, 0.49 mmol) and 4-chloro-3-(trifluoromethyl)benzylamine (43 μL, 0.28 mmol) in dichloromethane (1 mL) for 66.5 hours. Solvent for column chromatography: petroleum ethers/ethyl acetate (1:2). A colorless solid was obtained (80 mg, 0.14 mmol, 70%). M.p.: 200–202 °C; IR (KBr): 3390 cm^{-1} (N–H), 3290 cm^{-1} (N–H, br), 1645 cm^{-1} (C=O, br); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.49 (s, 1H, indole NH), 8.47 (t, J = 6.0 Hz, 1H, amide NH), 8.40 (t, J = 5.8 Hz, 1H, amide NH), 7.83–7.77 (m, 3H, ArH), 7.75 (d, J = 2.1 Hz, 1H, ArH), 7.64 (d, J = 8.3 Hz, 1H, ArH), 7.59–7.53 (m, 1H, ArH), 7.53–7.45 (m, 2H, ArH), 7.44–7.37 (m, 1H, ArH), 7.33 (dd, J = 8.5, 0.5 Hz, 1H, ArH), 7.21 (dd, J = 8.5, 2.0 Hz, 1H, ArH), 4.37 (d, J = 6.0 Hz, 2H, $-CH_2-$), 3.79 (d, J = 5.8 Hz, 2H, $-CH_2-$), 3.68 (s, 2H, $-CH_2-$); ^{13}C NMR (126 MHz, DMSO) δ 31.4, 41.1, 42.4 (CH_2), 113.0, 121.2, 124.0, 126.5 (q, J = 5.3 Hz), 127.9, 128.2 (2C), 128.7 (2C), 131.5, 132.8, (CH), 105.6, 111.4, 122.9 (q, J = 273.3 Hz), 124.4 (q, J = 30.7 Hz), 128.9 (q, J = 1.7 Hz), 130.7, 131.9, 134.5, 137.4, 139.7, 169.3, 171.1 (C); $C_{26}H_{20}BrClF_3N_3O_2$ (578.81); MS (ESI): m/z (%): pos.: 600.1

$[M+Na]^+$ (100), neg.: 576.1 $[M-H]^-$ (100); HPLC (isocr.): 98.0% at 254 nm, 98.3% at 280 nm, t_{M+S} = 4:43 min, t_M(DMSO) = 1.12 min (MeCN/water = 60:40); HPLC (grad.): 98.8% at 254 nm, t_{M+S} = 11.88 min, t_M(DMSO) = 1.14 min; λ_{max}: 225 nm, 309 nm; HR-MS (ESI): m/z $[M+Na]^+$ calc. 600.02717, found 600.02760.

2-[2-(1*H*-Indole-3-yl)acetamido]-*N*-(3,4-dichlorobenzyl)acetamide (7a). According to General Procedure A from [2-(1*H*-indole-3-yl)acetyl]glycine (**3b**, 84 mg, 0.36 mmol), EDC·HCl (104 mg, 0.543 mmol), DIPEA (0.09 mL, 0.52 mmol) and 3,4-dichlorobenzylamine (0.08 mL, 0.53 mmol) in acetonitrile (1 mL) for 69 hours. Deviating from General Procedure A, the compound has been purified by column chromatography twice. Solvents for column chromatography: 1. ethyl acetate/acetone (4:1), 2. Ethyl acetate. A pale yellow solid was obtained (68 mg, 0.16 mmol, 45%). M.p.: 55–57 °C; IR (KBr): 3397 cm^{-1} (N–H), 3285 cm^{-1} (N–H, br), 1650 cm^{-1} (C=O, br); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.86 (s, 1H, indole NH), 8.37 (t, J = 6.0 Hz, 1H, amide NH), 8.12 (t, J = 5.8 Hz, 1H, amide NH), 7.57–7.52 (m, 2H, ArH), 7.50 (d, J = 2.0 Hz, 1H, ArH), 7.33 (dt, J = 8.1, 1.0 Hz, 1H, ArH), 7.25–7.20 (m, 2H, ArH), 7.09–7.02 (m, 1H, ArH), 6.99–6.92 (m, 1H, ArH), 4.27 (d, J = 6.0 Hz, 2H, –CH_2–), 3.73 (d, J = 5.8 Hz, 2H, –CH_2–), 3.58 (s, 2H, –CH_2–); ^{13}C NMR (126 MHz, DMSO) δ 32.5, 40.9, 42.3 (CH_2), 111.2, 118.3, 118.6, 120.9, 123.9, 127.4, 129.1, 130.3 (CH), 108.6, 127.2, 129.2, 130.8, 136.1, 140.7, 169.3, 171.2 (C); $C_{19}H_{17}Cl_2N_3O_2$ (390.26); MS (ESI): m/z (%): pos.: 411.8 $[M+Na]^+$ (100), neg.: 387.8 $[M-H]^-$ (100); HPLC (isocr.): 99.5% at 254 nm, 99.8% at 280 nm, t_{M+S} = 6.06 min, t_M(DMSO) = 1.09 min (MeCN/water = 40:60); HPLC (grad.): 99.0% at 254 nm, t_{M+S} = 10.01 min, t_M(DMSO) = 1.16 min; λ_{max}: 274 nm, 280 nm; HR-MS (ESI): m/z $[M+H]^+$ calc. 390.07706, found 390.07747, $[M+Na]^+$ calc. 412.05900, found 412.05945.

2-[2-(1*H*-indole-3-yl)acetamido]-*N*-[4-chloro-3-(trifluoromethyl)-benzyl]acetamide (7b). According to General Procedure A from [2-(1*H*-indole-3-yl)acetyl]glycine (**3b**, 79 mg, 0.34 mmol), EDC·HCl (94 mg, 0.49 mmol), DIPEA (0.09 mL, 0.52 mmol) and 4-chloro-3-(trifluoromethyl)benzylamine (0.08 mL, 0.53 mmol) in acetonitrile (1 mL) for 69 hours. Solvent for column chromatography: ethyl acetate/acetone (4:1). A pale yellow solid was obtained (96 mg, 0.23 mmol, 67%). M.p.: 62–64 °C; IR (KBr): 3400 cm^{-1} (N–H, br), 1654 cm^{-1} (C=O, br); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.86 (s, 1H, indole NH), 8.43 (t, J = 6.1 Hz, 1H, amide NH), 8.13 (t, J = 5.8 Hz, 1H, amide NH), 7.73 (d, J = 2.1 Hz, 1H, ArH), 7.64 (d, J = 8.3 Hz, 1H, ArH), 7.57–7.51 (m, 2H, ArH), 7.39–7.31 (m, 1H, ArH), 7.22 (d, J = 2.4 Hz, 1H, ArH), 7.09–7.02 (m, 1H, ArH), 6.99–6.92 (m, 1H, ArH), 4.34 (d, J = 6.0 Hz, 2H, –CH_2–), 3.73 (d, J = 5.8 Hz, 2H, –CH_2–), 3.58 (s, 2H, –CH_2–); ^{13}C NMR (126 MHz, DMSO) δ 32.5, 41.1, 42.3 (CH_2), 111.3, 118.3, 118.6, 120.9, 123.9, 126.4 (q, J = 30.5 Hz), 131.5, 132.8 (CH), 108.6, 122.9 (q, J = 273.0 Hz), 126.5 (q, J = 5.2 Hz), 127.2, 128.9 (q, J = 2.1 Hz), 136.1, 139.7, 169.4, 171.2 (C); $C_{20}H_{17}ClF_3N_3O_2$ (423.82); MS (ESI): m/z (%): pos.: 445.8 $[M+Na]^+$ (100), neg.: 421.8 $[M-H]^-$ (100); HPLC (isocr.): 97.7% at 254 nm, 99.0% at 280 nm, t_{M+S} = 7.99 min, t_M(DMSO) = 1.08 min (MeCN/water = 40:60); HPLC (grad.): 97.5% at 254 nm, t_{M+S} = 10.31 min, t_M(DMSO) = 1.16 min; λ_{max}: 273 nm, 279 nm; HR-MS (ESI): m/z $[M+H]^+$ calc. 424.10342, found 424.10394, $[M+Na]^+$ calc. 446.08536, found 446.08585.

***N*-(3,4-Dichlorobenzyl)-2-[2-(5-methoxy-1*H*-indole-3-yl)-acetamido]acetamide (7c)**. According to General Procedure A from *N*-[2-(5-methoxy-1*H*-indole-3-yl)acetyl]glycine (**3c**, 96 mg, 0.37 mmol), EDC·HCl (100 mg, 0.523 mmol), DIPEA (0.09 mL, 0.52 mmol) and 3,4-dichlorobenzylamine (0.07 mL, 0.53 mmol) in acetonitrile (2 mL) for 64 hours. Deviating from General Procedure A, the compound has been purified by column chromatography twice. Solvents for column chromatography: 1. ethyl acetate/ethanol (20:1), 2. ethyl acetate/acetone (5:1). A colorless solid was obtained (126 mg, 0.300 mmol, 82%). M.p.: 124–127 °C; IR (KBr): 3408 cm^{-1} (N–H), 3278 cm^{-1} (N–H, br), 1681 cm^{-1} (C=O), 1626 cm^{-1} (C=O, br); ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.70 (s, 1H, indole NH), 8.39 (t, J = 6.0 Hz, 1H, amide NH), 8.12 (t, J = 5.8 Hz, 1H, amide NH), 7.54 (d, J = 8.3 Hz, 1H, ArH), 7.51–7.48 (m, 1H, ArH), 7.25–7.20 (m, 2H, ArH), 7.20–7.16 (m, 1H, ArH), 7.08–7.04 (m, 1H, ArH), 6.70 (ddd, J = 8.8, 2.5, 0.4 Hz, 1H, ArH), 4.26 (d, J = 6.0 Hz, 2H, –CH_2–), 3.74 (m, 5H, –CH_2–, –CH_3), 3.54 (d, J = 0.9 Hz, 2H, –CH_2–); ^{13}C NMR (151 MHz, DMSO-d_6) δ 55.3 (CH_3), 32.5, 40.9, 42.2 (CH_2), 100.6, 111.1, 111.9, 124.6, 127.4, 129.1, 130.4 (CH), 108.4, 127.5, 129.2, 130.8, 131.2, 140.7, 153.0, 169.3, 171.2 (C); $C_{20}H_{19}Cl_2N_3O_3$ (420.29); MS (ESI): m/z (%): pos.: 442.4 $[M+Na]^+$ (100), neg.: 418.4 $[M-H]^-$ (100); HPLC (isocr.): 98.6% at 254 nm, 99.6% at 280 nm, t_{M+S} = 5.44 min, t_M(DMSO) = 1.11 min (MeCN/water = 40:60); HPLC (grad.): 99.0% at 254 nm, t_{M+S} = 9.87 min, t_M(DMSO) = 1.15 min; λ_{max}: 274 nm; HR-MS (ESI): m/z $[M+H]^+$ calc. 420.08762, found 420.08807, $[M+Na]^+$ calc. 442.06957, found 442.07008.

***N*-[4-Chloro-3-(trifluoromethyl)benzyl]-2-[2-(5-methoxy-1*H*-indole-3-yl)acetamido]acetamide (7d)**. According to General Procedure A from *N*-[2-(5-methoxy-1*H*-indole-3-yl)acetyl]glycine (**3c**, 101 mg, 0.385 mmol), EDC·HCl (106 mg, 0.552 mmol), DIPEA (0.10 mL, 0.57 mmol) and 4-chloro-3-(trifluoromethyl)benzylamine (0.08 mL, 0.53 mmol) in acetonitrile (2 mL) for 60 hours. Solvent for column chromatography: ethyl acetate/ethanol (40:1). A colorless solid was obtained (151 mg, 0.332 mmol, 86%). M.p.: 122–124 °C; IR (KBr): 3409 cm^{-1} (N–H), 3297 cm^{-1} (N–H), 1649 cm^{-1} (C=O, br); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.71 (s, 1H, indole NH), 8.45 (t, J = 6.0 Hz, 1H, amide NH), 8.14 (t, J = 5.8 Hz, 1H, amide NH), 7.73 (d, J = 2.1 Hz, 1H, ArH), 7.64 (d, J = 8.3 Hz, 1H, ArH), 7.57–7.50 (m, 1H, ArH), 7.22 (dd, J = 8.8, 0.5 Hz, 1H, ArH), 7.18 (d, J = 2.5 Hz, 1H, ArH), 7.06 (d, J = 2.5 Hz, 1H, ArH), 6.70 (dd, J = 8.7, 2.4 Hz, 1H, ArH), 4.34 (d, J = 6.0 Hz, 2H, –CH_2–), 3.77–3.71 (m, 5H, –CH_2–, –CH_3), 3.54 (s, 2H, –CH_2–);^{13}C NMR (101 MHz, DMSO) δ 55.3 (CH_3), 32.5, 41.1, 42.2 (CH_2), 100.6, 111.0, 111.9, 124.5,, 126.5 (q, J = 5.3 Hz), 131.4, 132.8 (CH), 108.3, 122.9 (q, J = 273.0 Hz), 126.4 (q, J = 30.7 Hz), 127.5, 128.8 (q, J = 1.8 Hz), 131.2, 139.7, 153.0, 169.4, 171.2 (C); $C_{21}H_{19}ClF_3N_3O_3$ (453.85); MS (ESI): m/z (%): pos.: 476.1 $[M+Na]^+$ (100), 303.1 $[M-150]^+$ (46), neg.: 452.2 $[M-H]^-$ (100); HPLC (isocr.): 98.2% at 254 nm, 99.6% at 280 nm, t_{M+S} = 7.15 min, t_M(DMSO) = 1.12 min (MeCN/water = 40:60); HPLC (grad.): 97.6% at 254 nm, t_{M+S} = 10.00 min, t_M(DMSO) = 1.17 min; λ_{max}: 274 nm; HR-MS (ESI): m/z $[M+H]^+$ calc. 454.11398, found 454.11475, $[M+Na]^+$ calc. 476.09592, found 476.09672.

4-[2-(5-Bromo-1*H*-indole-3-yl)acetyl]-1-(3,4-dichlorobenzyl)-piperazine-2-one (14a). According to General Procedure A from 1-(3,4-dichlorobenzyl)piperazine-2-one (**13**, 62 mg, 0.24 mmol), EDC·HCl (55 mg, 0.29 mmol), DIPEA (0.07 mL, 0.40 mmol) and 2-(5-bromo-1*H*-indole-3-yl)acetic acid (71 mg, 0.28 mmol) in acetonitrile (1.5 mL) for 47 hours. Solvent for column chromatography: ethyl acetate/ethanol (20:1). A pale yellow solid was obtained (74 mg, 0.15 mmol, 62%). M.p.: 175 °C (decomp.); IR (KBr): 3424 cm^{-1} (N–H, br), 1641 cm^{-1} (C=O, br); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.11 (br s, 1H, indole NH), 7.75–7.70 (m, 1H, ArH), 7.59–7.53 (m, 1H, ArH), 7.51 (d, J = 2.0 Hz, 1H, ArH), 7.34–7.27 (m, 2H, ArH), 7.21–7.13 (m, 2H, ArH), 4.50 (s, 2.0H, –CH_2–), 4.29 (s, 0.8H, –CH_2), 4.11 (s, 1.1H, –CH_2), 3.82–3.77 (m, 3.1H, –CH_2–), 3.71–3.66 (m, 0.9H, –CH_2–), 3.26–3.21 (m, 2H, –CH_2–), 2.09 (s, 2H, –CH_2–) (isomeric ratio: ca. 4:3); ^{13}C NMR (126 MHz, DMSO) δ 30.0, 30.2, 38.4, 42.4, 45.4, 45.8, 45.9, 47.9, 48.1, 48.9 (CH_2), 113.3, 121.1, 121.2, 123.5, 125.4, 125.5, 127.8, 127.9, 129.7, 129.8, 130.7 (CH), 107.5, 107.7, 111.1, 129.1, 129.1 129.8, 131.1, 134.8, 138.2, 164.8, 165.4, 169.0 (C); $C_{21}H_{18}BrCl_2N_3O_2$ (495.20); MS (ESI): m/z (%): pos: 516.0 $[M+Na]^+$ (30), neg: 492.1 $[M-H]^-$ (100); HPLC (isocr.): 94.3% at 254 nm, 97.3% at 280 nm, t_{M+S} = 6.15 min, t_M(DMSO) = 1.12 min (MeCN/water = 50:50); HPLC (grad.): 96.2% at 254 nm, t_{M+S} = 11.06 min, tM(DMSO) = 1.17 min; λ_{max}: 221 nm, 238 nm, 282 nm, 289 nm; HR-MS (ESI): m/z $[M+H]^+$ calc.

494.00322, found 494.00294, $[M+Na]^+$ calc. 515.98517, found 515.98522.

4-[2-(5-Methoxy-1*H*-indole-3-yl)acetyl]-1-(3,4-dichlorobenzyl)-piperazine-2-one (14 b). According to General Procedure A from 1-(3,4-dichlorobenzyl)piperazine-2-one (**13**, 88 mg, 0.34 mmol), EDC·HCl (87 mg, 0.45 mmol), DIPEA (0.10 mL, 0.57 mmol) and 2-(5-methoxy-1*H*-indole-3-yl)acetic acid (86 mg, 0.42 mmol) in acetonitrile (1.5 mL) for 47 hours. Solvent for column chromatography: ethyl acetate/ethanol (20:1). A pale yellow solid was obtained (125 mg, 0.279 mmol, 82%). M.p.: 177 °C (decomp.); IR (KBr): 3417 cm^{-1} (N–H, br), 1640 cm^{-1} (C=O); ^1H NMR (500 MHz, DMSO-d_6) δ 10.76–10.73 (m, 1H, indole NH), 7.58–7.52 (m, 1H, ArH), 7.52–7.48 (m, 1H, ArH), 7.27–7.19 (m, 1H, ArH), 7.19–7.15 (m, 1H, ArH), 7.18–7.09 (m, 1H, ArH), 7.06 (d, J = 2.5 Hz, 0.4H, ArH), 7.03 (d, J = 2.4 Hz, 0.6H, ArH), 6.76–6.69 (m, 1H, ArH), 4.51–4.47 (m, 2H, $-CH_2-$), 4.28 (s, 0.8H, $-CH_2-$), 4.12 (s, 1.1H, $-CH_2-$), 3.81–3.67 (m, 7H, $-CH_2-$, $-CH_3$), 3.26–3.21 (m, 0.8H, $-CH_2-$), 3.21–3.16 (m, 1.3H, $-CH_2-$) (isomeric ratio: ca. 3:2); ^{13}C NMR (126 MHz, DMSO) δ 55.3 (CH_3), 30.5, 30.7, 38.4, 42.5, 45.4, 45.8, 45.9, 47.8, 48.0, 49.0 (CH_2), 100.5, 100.7, 111.2, 112.0, 124.2, 124.2, 127.7, 127.8, 129.8, 130.7 (CH), 107.2, 107.3, 127.4, 127.5, 129.8, 131.1, 131.2, 131.3, 138.2, 138.2, 153.0, 164.9, 165.4, 169.3 (C); $C_{22}H_{21}Cl_2N_3O_3$ (446.33); MS (ESI): m/z (%): pos: 468.1 $[M+Na]^+$ (17), neg: 444.2 $[M-H]^-$ (100); HPLC (isocr.): 98.1% at 254 nm, 99.3% at 280 nm, t_{M+S} = 3.51 min, t_M(DMSO) = 1.12 min (MeCN/water = 50:50); HPLC (grad.): 99.4% at 254 nm, t_{M+S} = 10.19 min, t_M(DMSO) = 1.17 min; λ_{max}: 221 nm, 234 nm, 275 nm; HR-MS (ESI): m/z $[M+H]^+$ calc. 446.10327, found 446.10366, $[M+Na]^+$ calc. 468.08522, found 468.08589.

Biology

Expression and purification of recombinant TryS

The recombinant form of His-tagged TryS from *L. infantum* and *T. b. brucei* was expressed in *Escherichia coli* strain BL-21 (DE-3) and purified as described in detail in [12].

TryS activity screening assays

The activity assays were conducted at nearly physiological substrates' concentrations for both TryS: 150 μM ATP, 2 mM Spd and 0.05 mM or 0.25 mM GSH for *Tb*TryS and *Li*TryS, respectively.[12] A master mix (MM) solution containing all the substrates at 1.25-fold their end concentration in the assay was prepared in screening reaction buffer (5 mM DTT, 10 mM $MgSO_4$, 0.5 mM EDTA, 100 mM HEPES pH 7.4, 9 mM NaCl, 10% DMSO (v/v)) and kept on ice until use. Microtiter plate wells were loaded with 5 μL of test compounds, DMSO (reaction control) or TryS specific inhibitor (MOL2008 at its IC_{50} for *Tb*TryS (30 μM) and *Li*TryS (150 nM); inhibition control) and 40 μL of MM. The reactions were started by adding 5 μL of TryS (2 or 3×10^{-5} μmol/(min×mL) for *Li*TryS or *Tb*TryS, respectively) and stopped after 15 min with 200 μL BIOMOL® GREEN reagent (Enzo Life Sciences, Lörrach, Germany). Blanks were prepared for each condition by adding 5 μL of screening reaction buffer instead of enzyme. The TryS activity was calculated according to the following formula:

$$\%\ TryS\ activity = \frac{A_{650nm\ Ci} - A_{650nm\ CiB}}{A_{650nm\ E} - A_{650nm\ EB}} \times 100\,\% \quad (1)$$

where $A_{650\,nm}$ refers to the mean absorbances at 650 nm corresponding to the reaction test with compound i (Ci), the blank control with compound i (CiB), the reaction control with DMSO (E) and the blank control with DMSO (EB). The errors are expressed as two standard deviations (SD). SD was estimated as σ×(n-1).

Viability assays for bloodstream T. b. brucei

The bloodstream form of the monomorphic *T. b. brucei* strain 427 (cell line 514–1313) expressing an ectopic copy of the *Photinus pyralis* red-shifted Luciferase,[23] was cultured axenically in HMI-9 medium,[36] supplemented with 10% fetal bovine serum, 10 U/mL penicillin (Gibco, ThermoFisher Scientific, Waltham, MA, USA), 10 μg/mL streptomycin (Gibco), 0.2 μg/mL bleomycin (Jena Bioscience, Jena, Germany), 4 μg/mL G418 disulfate salt (Sigma-Aldrich, St. Louis, MO, USA) and 0.2 μg/mL puromycin (Invitrogen, ThermoFisher Scientific, Waltham, MA, USA), inside a humidified incubator (ThermoFisher Scientific, Waltham, MA, USA) with controlled temperature (37 °C) and CO_2 (5%) in 25 or 75 cm^2 vented-cap culture-flask.

The anti-trypanosomal activity of the compounds was evaluated against the cell line mentioned above by quantifying the bioluminescence signal, which is proportional to parasite number and metabolic state.[23] Briefly, in a 96-well culture plate containing 2.2 μL/well DMSO (negative control) or nifurtimox (5 uM, positive control) or compounds dissolved in DMSO (1% final concentration), 220 μL/well of a suspension of 1×10^5 exponentially growing parasites/mL was added. The plates were incubated at 37 °C and 5% CO_2 for 24 h. Next, the samples were transferred to a 96-well black plate and 20 μL/well of a solution containing D-Luciferin (1.5 mg/mL in 1% PBS glucose (w/v)) and 0.05% Triton X-100 (v/v) was added. The bioluminescence signal was measured in a LUMIstar OPTIMA Microplate luminometer using the following settings: 10 s shaking, 5 s/well acquisition, 0.2 s measurement delay, maximum gain, and 37 °C. Parasite viability was calculated according to the following formula:

$$\%\ parasite\ viability = \frac{BL_{Ci} - BL_{blank}}{BL_{neg} - BL_{blank}} \times 100\,\%, \quad (2)$$

where *BL* refers to the mean of bioluminescence signal corresponding to the tested compound i (Ci), the blank (blank; complete media containing 1% DMSO (v/v)), or the negative control (neg; parasites treated with 1% DMSO (v/v)). EC_{50} values were determined from concentration-response curves fitted to a four-parameter sigmoid equation using the GraphPad Prism software (version 6.0). All errors are expressed as one SD. For a detailed explanation of the assay protocol see.[25]

Viability assays for murine macrophages

Murine macrophages from the cell line J774 (ATCC® TIB-67TM) were cultivated under a humidified 5% CO_2/95% air atmosphere at 37 °C in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS (v/v), 10 U/mL penicillin and 10 μg/mL streptomycin. The cytotoxicity of the compounds was evaluated using the WST-1 reagent (Roche, Basel, Switzerland) and almost the same experimental protocol described previously,[37] except that 200 μL/well of a cell suspension at 6×10^4 cells/mL was added in a 96-well culture plate and upon 24 h incubation with compounds, the cell monolayer was washed with 150 μL of DMEM. The CC_{50} against macrophages was assayed only for compounds for which the corresponding EC_{50} towards parasites was determined and did not show solubility issues within the concentration range of interest. Control treatments included cells cultured in the presence of 1% DMSO (v/v). Absorbance at 450 nm ($A_{450\,nm}$), corresponding to the formazan dye produced by metabolically active cells, was measured

with an EL 800 microplate800-microplate reader. The corrected absorbance values at 450 nm ($A_{corr\,i\,450\,nm}$) were obtained by subtracting the corresponding absorbance value at 630 nm and the blank average from the obtained absorbance values at 450 nm ($A_{i\,450\,nm}$), e.g.:

$$A_{corr\ i\ 450\ nm} = A_{i\ 450\ nm} - A_{i\ 630\ nm} - A_{blank\ 450\ nm} \quad (3)$$

The absorbance is proportional to the number of surviving cells and cell viability was calculated according to the following formula:

$$\%\ cell\ viability = \frac{A_{corr\ i\ 450\ nm}}{A_{corr\ negative\ control\ 450\ nm}} \times 100\,\% \quad (4)$$

where $A_{corr\ i\ 450\ nm}$ and $A_{corr\ negative\ control\ 450\ nm}$ refer to cells treated with compound i at a specific concentration (Ci) or with DMSO (negative control). CC_{50} values were obtained from the corresponding concentration-response plots fitted to a four-parameter sigmoid equation or extrapolated from nonlinear fitting equations. The errors were calculated using error propagations and are expressed as one SD.

Selectivity indices (SI) were calculated as the ratio of CC_{50} for murine macrophages (cell line J774) vs. EC_{50} for *T. b. brucei* parasites.

All assays were performed in triplicates.

Supporting Information

The Supporting Information contains biochemical and biological results, synthetic procedures and analytical characterization of precursors and NMR spectra as well as structures and data of analogues **4e**, **4f**, **4g**, **4l**, **5j**, **5k** which are not included in the main text.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: antiprotozoal agents · structure-activity relationships · indole · *Trypanosoma brucei* · Topliss approach

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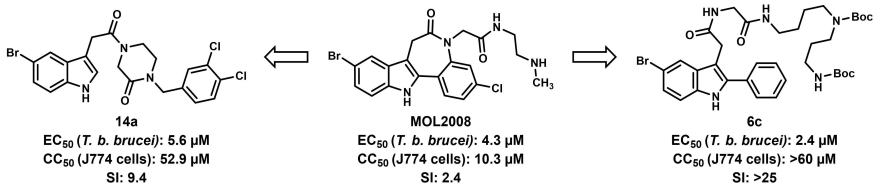

Treatment of Chagas disease, African sleeping sickness and Leishmaniasis is currently limited to few, unfavorable drugs. We discovered novel antitrypanosomal compounds *via* molecular simplification of an original molecule (MOL2008) displaying limited selectivity. The new compounds are less cytotoxic and qualify as hits for further development/optimization.

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Discovery of Antitrypanosomal Indolylacetamides by a Deconstruction–Optimization Strategy Applied to Paullones