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Improved proteolytic stability and potent activity against *Leishmania infantum* trypanothione reductase of α/β-peptide foldamers conjugated to cell-penetrating peptides

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Abbreviations- CPP, cell-penetrating peptide; DSSP, define secondary structure of proteins algorithm; ELISA, Enzyme-Linked ImmunoSorbent Assay; 5-FITC, fluorescein 5-isothiocyanate, HCTU, *O*-(6-chlorobenzotriazol-1-yl)-*1,1,3,3*-tetramethyluronium hexafluorophosphate, IC_{50}, inhibitory concentration 50; Li-TryR, *Leishmania infantum* trypanothione reductase; EC_{50}, effective concentration 50; MD, molecular dynamics; PPIs, protein-protein interaction inhibitors; PEG, polyethylene glycol, PI, propidium iodide; SI, selectivity index, SPPS, solid phase peptide synthesis, VL, visceral leishmaniasis.

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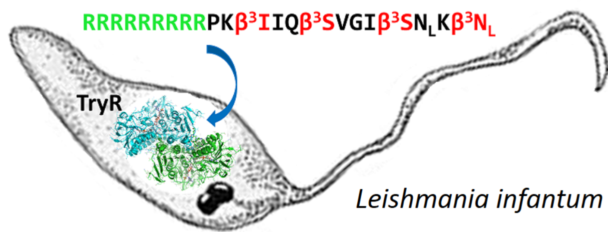
ABSTRACT— The objective of the current study was to enhance the proteolytic stability of peptide-based inhibitors that target critical protein-protein interactions at the dimerization interface of *Leishmania infantum* trypanothione reductase (*Li*-TryR) using a backbone modification strategy. To achieve this goal we carried out the synthesis, proteolytic stability studies and biological evaluation of a small library of α/β^3-peptide foldamers of different length (from 9-mers to 13-mers) and different $\alpha\rightarrow\beta$ substitution patterns related to prototype linear α-peptides. We show that several 13-residue α/β^3-peptide foldamers retain inhibitory potency against the enzyme (in both activity and dimerization assays) while they are far less susceptible to proteolytic degradation than an analogous α-peptide. The strong dependence of the binding affinities for Li-TryR on the length of the α,β-peptides is supported by theoretical calculations on conformational ensembles of the resulting complexes. The conjugation of the most proteolytically stable α/β-peptide with oligoarginines results in a molecule with potent activity against *L. infantum* promastigotes and amastigotes.

Keywords- α/β-peptides, foldamers, proteolysis, protein-protein interactions, trypanothione reductase, *Leishmania infantum*

Graphical abstract



Highlights

- α/β-peptide foldamers targeting the dimer interface of Li-TryR are described
- The length of the α/β-peptide is important for TryR inhibitory activities
- A substantial increase in proteolytic stability compared to the α-peptides is observed
- Conjugation to oligoarginines results in potent leishmanicidal activity

INTRODUCTION

Leishmaniasis is a severe neglected tropical disease caused by parasites belonging to the genus *Leishmania*. One of the most serious clinical forms of this disease is visceral leishmaniasis (VL), caused by *Leishmania donovani* and *Leishmania infantum*, which is invariably fatal if left untreated. According to World Health Organization (WHO), an estimated 200,000 to 400,000 new cases of VL and over 20,000 deaths occur worldwide each year [1]. The drugs available in clinical use, such as pentavalent antimony, paramomycin, amphotericin B and the newest and only oral drug for VL, miltefosine, show serious limitations including toxicity and drug resistance [2, 3]. There is clearly an urgent need for innovative drugs that act on new molecular targets and/or by new inhibition mechanisms.

Trypanothione reductase (TryR) is a validated drug target in trypanosomatids, as it is a crucial enzyme for the antioxidant defenses of these parasites and it is absent in humans [4, 5]. TryR is an NADPH-dependent flavoenzyme that combats oxidative stress by maintaining adequate levels of the reducing agent trypanothione (an unusual spermidine-glutathione conjugate). Another important characteristic of TryR is its significant structural difference from glutathione reductase (GR), the enzyme with the corresponding functions in humans. A range of structurally diverse inhibitors of TryR have been described [6-8] that are good trypanocides *in vitro* but there are scarce reports of compounds that are also effective *in vivo*. Based of the fact that the functional form of TryR is a homodimer, we have recently reported an alternative inhibition strategy directed at disrupting the dimer interface of *Leishmania infantum* TryR (Li-TryR) by means of peptide-based protein-protein interaction (PPI) inhibitors [9]. Potential druggable sites for disrupting PPIs were explored by a combination of molecular modelling and site-directed mutagenesis studies. From a small library of linear peptides of different length, derived from an α-helix spanning residues P435 to M447 that contained the identified hotspot E436, the 13-residue wild-peptide sequence PEIIQSVGIS-

Nle-K-Nle (**1**) and the modified peptide sequence PKIIQSVGIS-Nle-K-Nle (**2**), in which the E residue at position 2 was replaced by a K, emerged as potent Li-TryR dimerization inhibitors in the low micromolar range [9]. From a series of C-terminally truncated peptides, the linear 11-*mer* PEIIQSVGIS-Nle (**3**) maintains potent inhibition of TryR enzymatic activity while the 9-*mer* analogue PKIIQSVGI (**4**) represents the minimal length required for inhibition [9].

The use of peptides is hampered by their enzymatic degradation and low bioavailability. The most common strategies used to increase stability against proteases include peptide cyclization (e.g. stapled peptides) and introduction of D- or unnatural amino acids (e.g. β-peptides and peptoids) [10-12]. Moreover, short linear peptides also have low conformational stability, which could decrease binding to the target. Several strategies for the development of peptide-based PPI inhibitors revealed that these drawbacks of α-peptides could be effectively reduced [12].

Cyclization is one of the most widely used methods for developing PPI inhibitors. In order to stabilize the α-helical structure of the short linear "hit" peptides, we recently prepared amide- and hydrocarbon-bridged cyclic analogues of **1** and **2** that retained potent inhibitory enzymatic activity and showed moderate resistance against degradation by proteinase K (a promiscous non-specific serine protease) relative to the linear prototypes [13, 14]. Further conjugation of cyclic analogues of the more potent linear peptide **2** with cationic cell-penetrating peptides (e.g. oligoarginines and Tat) demonstrated, for the first time, an effective *in vitro* antileishmanial activity of TryR dimerization peptide-based inhibitors against *Leishmania infantum*. However, a certain degree of backbone flexibility seems to be necessary for keeping the inhibitory potency in the cyclic derivatives [14].

The objective of the current study was to develop proteolytically stable analogues of TryR dimerization-inhibiting peptides by introducing modifications on the backbone that allow

flexibility while maintaining potency against the enzyme and the parasites *in vitro*. To achieve this goal we focused on α-helix-mimetic oligomers (foldamers) [15] containing both α and unnatural β^3-amino acid residues (mixed α/β-peptides) following the sequence-based strategy described by Gellman and co-workers [16-18]. This approach involves replacing α residues with β^3 residues bearing the original side chains according to simple patterns periodically distributed along one side of the helix such as ααβαααβ, ααβ and αααβ. These replacements result in 25-33% β residue incorporation in contrast with previous foldamers with pure β backbones or 1:1 α/β residue alternation. Several studies showed that this sequence-based strategy can succesfully lead to formation of α-helix-like conformations and significantly disminishes susceptibility to degradation by proteases [19-22]. Moreover, backbone flexibility would be increased because the β^3-amino acids introduce an extra CH_2 unit.

With this background in mind, we undertook the synthesis, proteolytic stability studies and biological evaluation of a small library of α/β^3-peptides of different length (from 9-mers to 13-mers) and different α→β substitution patterns related to the prototype α-peptide **2**. Most of the 13-residue α/β^3-peptides retained significant potency against TryR (in both activity and dimerization assays) and some of them displayed substantially improved stability against proteinase K compared to the amide-bridge cyclic analogues of linear prototype α-peptide **2** [14]. Interestingly, the most proteolytically stable α/β^3-peptide of the series, when conjugated to oligoarginines acting as cell-penetrating peptides (CPP) showed potent activity *in vitro* against both promastigotic and amastigotic forms of *Leishmania infantum* and lower toxicity against monocytic THP-1 cells than previous CPP-conjugated linear and cyclic peptides.

RESULTS AND DISCUSSION

Design and Synthesis

The primary sequence of target α/β^3-peptides and the $\alpha \rightarrow \beta$ substitution patterns are depicted in Figure 1. These compounds retain the side-chain sequences of the 13-mer, 11-mer and 9-mer α-peptides **2-4** but the β^3-amino acid residues introduce an extra CH_2 unit. In particular, α/β^3-peptide foldamers **5-11** represent all isomers of the 13-mer α-peptide prototype **2** sequence with the ααβαααβ backbone pattern while α/β^3-peptides **12** and **13** share the αααβ backbone pattern. Similarly, the ααβαααβ pattern was also used for the design of examples of 11- and 9-residue α/β^3-peptides **14-18** (Figure 1).

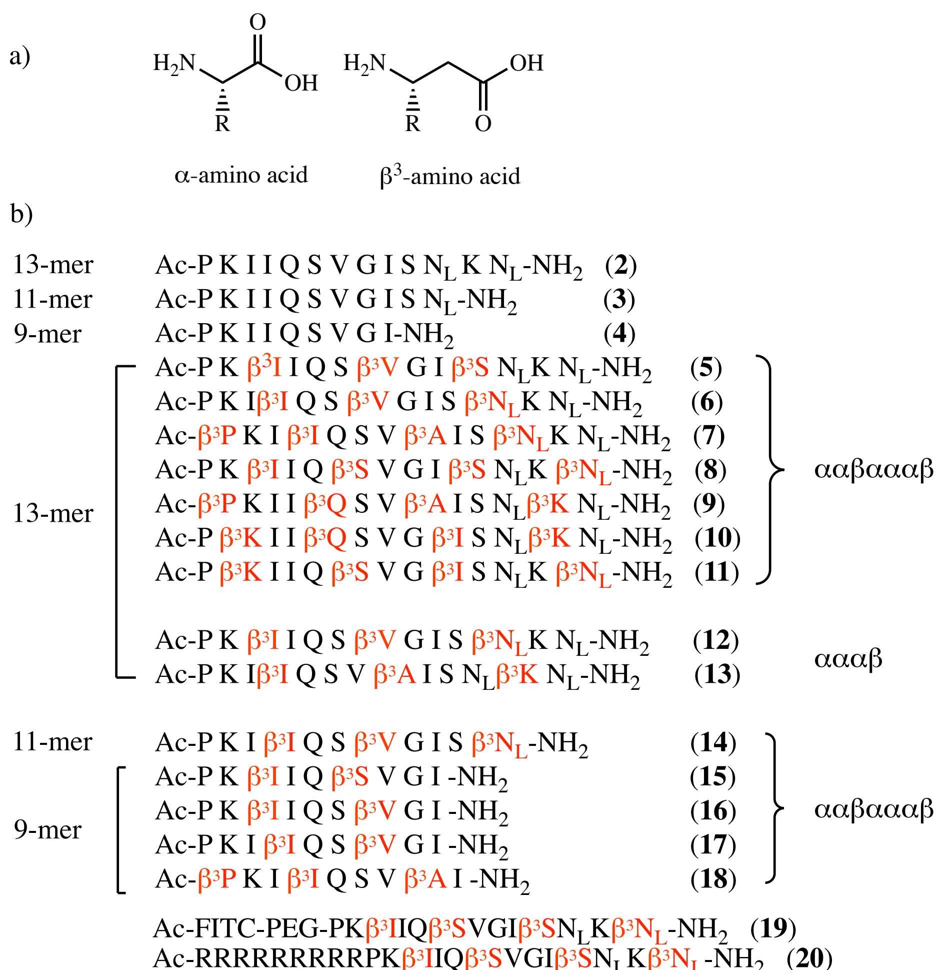


Figure 1. a) Structure of an α-amino acid and a β^3-amino acid; b) Sequences of α-peptides **2-4** derived from the *Li*-TryR interface domain, target α/β-peptide analogues **5-18** (13-mer, 11-mer and 9-mer), and FITC and R_9 conjugates **19** and **20**. β^3 residues are indicated in red. β^3 glycine residues have been replaced by commercially available β^3 alanine residues.

To visualize the cellular uptake of these molecules, the most proteolytically stable peptide (**8**) was labeled with fluorescein 5-isothiocyanate (5-FITC) through the N-terminus via a PEG spacer to enhance aqueous solubility (compound **19**). Finally, the α/β^3-peptide **8** was conjugated to oligoarginines (R_9) as cell-penetrating peptides (compound **20**) for *in vitro* evaluation against parasites (*L. infantum* promastigotes and axenic amastigotes) based on previous results with prototype **2** and cyclic peptide analogues [14].

The α/β-peptide library was prepared manually by microwave-assisted solid-phase peptide synthesis (SPPS) on a Rink amide MBHA polystyrene resin. The Fmoc-α-amino acids and Fmoc-β^3-amino acids were coupled using HCTU/DIEA in DMF at 40 °C under microwave conditions. After purification by Biotage or preparative RP-HPLC, compounds **5-18** were isolated in 11-49% overall yields. The stepwise SPPS procedures for the synthesis of FITC and oligoarginines conjugated compounds **19** and **20** are also detailed in the experimental section. The purities of α/β-peptides and conjugates were confirmed by RP-HPLC and HRMS. The analytical data, purities and overall yields of compounds are indicated in Table S1.

Proteolytic susceptibility

We examined the impact of nonnatural amino acid incorporation on the susceptibility to degradation by proteinase K, a promiscous non-specific serine protease (Table 1). The 13-mer linear α-peptide **2** is rapidly degraded, with a half-life of 23 min under the assay conditions. In contrast, the half-life of α/β-peptide **8** is 517 min, > 22-fold longer than the half-life for cleavage of the α-peptide. This increase in stability is not universal among the series: whereas α/β-peptides **7**, **9** and **12** do not show any improvement in stability over the α-peptide, the half-lives of compounds **6**, **10** and **11** is 6- to 14-fold higher relative to **2**. Thus, the precise positioning of α→β replacements is important with regard to proteolytic susceptibility.

Table 1. Proteolysis of α-peptide **2** and α/β-peptide analogues **6-12**

α- or α/β-peptide	$t_{1/2}$ (min)[a]
2	23
6	215
7	23
8	517
9	< 15
10	317
11	131
12	< 15

[a] Half-life of α- and α/β-peptides (50 μM) in the presence of proteinase K (10 μg/mL) in pH 7.6. Remaining peptide was graphed *vs* time and fit to a simple exponential decay equation to obtain a half-life in GraphPad Prism4.

These observations are consistent with previous findings for oligomers and suggest that incorporation of 25-30% β^3 residues using this backbone pattern can lead to significant improvements in half-life in the presence of proteinase K [16-18, 21, 22].

Interestingly, α/β-peptide **8** displayed a 8-10-fold- higher proteolytic resistance relative to our previously described lactam-bridged α-peptide analogues ($t_{1/2}$ 517 min *vs* 52 or 64 min) [14].

Biological activity

Enzymatic assays

All the synthetized compounds were evaluated as Li-TryR inhibitors [23] (Table 2). Peptide length showed to be more relevant in the α/β-peptide analogues than in their α-prototypes. Thus, all short α/β 11-mers and 9-mers (**14** and **15-18** respectively) were less active against Li-TryR than the α-peptides **3** and **4.** Even though most of the 13-residue foldamer analogues (**5-13)** showed relevant inhibitory activity, IC_{50} values similar to that of prototype **2** were only found in peptides with the ααβαααβ backbone pattern (**6**, **7**, **9** and **11**).

In general terms, α/β-peptide analogues were slightly less active in the dimerization assay than prototype **2** and, as expected, the compouds found to be the most active in the enzymatic assay (**6**, **7** and **9**) were also the best dimerization disruptors in our ELISA.

Table 2. IC_{50} ± SEM values for the α/β-peptide analogues **5-18** in the oxidoreductase activity and the Li-TryR monomer displacement assays.

Peptide[a]	**IC_{50} activity (μM)**[b]	**IC_{50} dimerization (μM)**[c]
2 (linear 13-mer prototype) **TRL35**	1.2 ± 0.17	10.35 ± 2.93
3 (linear 11-mer prototype) **TRL34 (2)**	0.82 ± 0.003	4.21 ± 0.23
4 (linear 9-mer prototype) **TRL38**	3.59 ± 0.27	15.74 ± 0.56
5 (Ac-PKβ^3IIQSβ^3VGIβ^3SN$_L$KN$_L$-NH$_2$) 13-mer	8.78 ± 1.1	13.71 ± 1.38
6 (Ac-PKIβ^3IQSβ^3VGISβ^3N$_L$KN$_L$-NH$_2$) 13-mer	3.11 ± 0.17	10.3 ± 1.26
7(Ac-β^3PKIβ^3IQSVβ^3AISβ^3N$_L$KN$_L$-NH$_2$) 13-mer	2.69 ± 0.95	13.95 ± 0.59
8 (Ac-PKβ^3IIQβ^3SVGIβ^3SN$_L$Kβ^3N$_L$-NH$_2$) 13-mer	3.47 ± 0.26	21.68 ± 1.3
9 (Ac-β^3PKIIβ^3QSVβ^3AISN$_L$$\beta^3KN_L$-NH$_2$) 13-mer	1.65 ± 0.43	12.82 ± 0.83
10 (Ac-Pβ^3KIIβ^3QSVGβ^3ISN$_L$$\beta^3KN_L$-NH$_2$) 13-mer	9.28 ± 1.16	15.48 ± 0.83
11 (Ac-Pβ^3KIIQβ^3SVGβ^3ISN$_L$Kβ^3N$_L$-NH$_2$) 13-mer	2.08 ± 0.78	18.04 ± 1.38
12 (Ac-PKβ^3IIQSβ^3VGISβ^3N$_L$KN$_L$-NH$_2$) 13-mer	18.16 ± 2.31	9.72 ± 1.12
13 (Ac-PKIβ^3IQSVβ^3AISN$_L$$\beta^3KN_L$-NH$_2$) 13-mer	5.38 ± 1.97	13.75 ± 1.09
14 (Ac-PKIβ^3IQSβ^3VGISβ^3N$_L$-NH$_2$) 11-mer	28.46 ± 3.51	47.3 ± 4.31
15 (Ac-PKβ^3IIQβ^3SVGI-NH$_2$) 9-mer	> 75	22.26 ± 0.62
16 (Ac-PKβ^3IIQSβ^3VGI-NH$_2$) 9-mer	> 75	53.04 ± 4.66
17 (Ac-PKIβ^3IQSβ^3VGI-NH$_2$) 9-mer	> 75	41.84 ± 1.48
18 (Ac-β^3PKIβ^3IQSVβ^3AI-NH$_2$) 9-mer	39.77 ± 4.92	50.4 ± 0.0

[a] β^3 residues are indicated in red. [b] Enzymatic activity > 75 indicates that the IC_{50} is higher than 75 μM (maximum assayed). Results are representative of three independent experiments each performed in triplicate. [c] Dimer quantitation assay (ELISA) [9].

Anti-leishmanial activity in cell culture

All the α/β-peptides were tested *in vitro* against *L. infantum* promastigotes and axenic amastigotes using edelfosine and miltefosine as positive controls [24]. None of them displayed any significant activity at the maximum concentration assayed (25 μM). Previous results from our group demonstrated that the cationic CPP composed of 9 arginines (R_9) facilitated passage through the plasma membrane and converted peptide prototypes into leishmanicidal agents [14]. Linkage of the R_9 sequence to the *N*-terminus of α/β-peptide foldamer **8** (compound **20**) converted our α/β-peptide into a leishmanicidal agent showing an EC_{50} value against amastigotes similar or even better to that observed for edelfosine and miltefosine (Table 3). α/β-peptide **8** was selected for R_9 ligation because of its high proteolytic stability and good IC_{50} value in the enzyme inhibition assay.

Table 3. LC_{50} ± SEM values for the [CPP]-[α/β-peptide foldamer] conjugate **20**, R_9 sequence (**21**) and linear and cyclic [CPP]-[α-peptide] conjugates on *L. infantum* promastigotes, amastigotes and cytotoxic activity in THP-1 cell line.[a]

Compound[b]	LC_{50} (μM) Promastigotes	LC_{50} (μM) Amastigotes	EC_{50} (μM) THP-1	SI[d]
20 RRRRRRRRRPKβ^3IIQβ^3SVGIβ^3SN$_L$Kβ^3N$_L$	0.98 ± 0.02	1.9 ± 0.5	5.8 ± 1.4	5.9 / 3.1
21 RRRRRRRRR	> 25	> 25	> 25	-
22 RRRRRRRRRPKIIQSV AISN$_L$KN$_L$[c]	4.6 ± 0.2	3.5 ± 0.9	1.7 ± 0.5	<1 / <1
23 RRRRRRRRRPK-*c*[$(CH_2)_2CONH(CH_2)_4$]^{3,7} [EIQSK]GISN$_L$KN$_L$[c]	3.5 ± 0.4	2.8 ± 0.3	2.5 ± 0.8	<1 / <1
Edelfosine	9 ± 0.2	0.6 ± 0.1	1 ± 0.2	< 1 / 1.7
Miltefosine	47.6 ± 0.6	2 ± 0.1	19 ± 1.6	<1 / 9.5

[a] Results are representative of three independent experiments each performed in triplicate.
[b] β^3 residues are indicated in red. All the peptides are acylated and as carboxamides at the *N*-terminal and *C*-terminal end, respectively. [c] LC_{50} values of linear and cyclic α-peptide conjugates are taken from reference 14.[d] Selectivity index (SI) is the ratio EC_{50} values of compounds against THP-1 cells relative to their corresponding EC_{50} values against promastigotes and amastigotes, respectively.

Remarkably, no significant leishmanicidal activity was observed upon incubation of the parasites with non-conjugated R_9 (compound **21**). The increase in EC_{50} values against *L. infantum* promastigotes and amastigotes compared to that of the R_9-conjugated version of α-linear peptide prototype (compound **22)** and lactam-bridged α-peptide analogues (compound **23**) validates our strategy for the design of α/β-peptides as TryR inhibitors. Notably, the presence of unnatural β^3-amino acid residues not only increased the potency of our peptides as leishmanicidal agents but also decreased their toxicity against THP-1 cells resulting in better selectivity indexes (Table 3).

Molecular modeling

The impact of α/β-peptide foldamer length on the computed binding energy with Li-TryR was evaluated by means of classical MD simulations and theoretical calculations (Figure 2). Figure 2 shows the binding energy of three representative α/β-peptide foldamers (**6**, **14** and **17**) that share a common sequence and differ in size only.

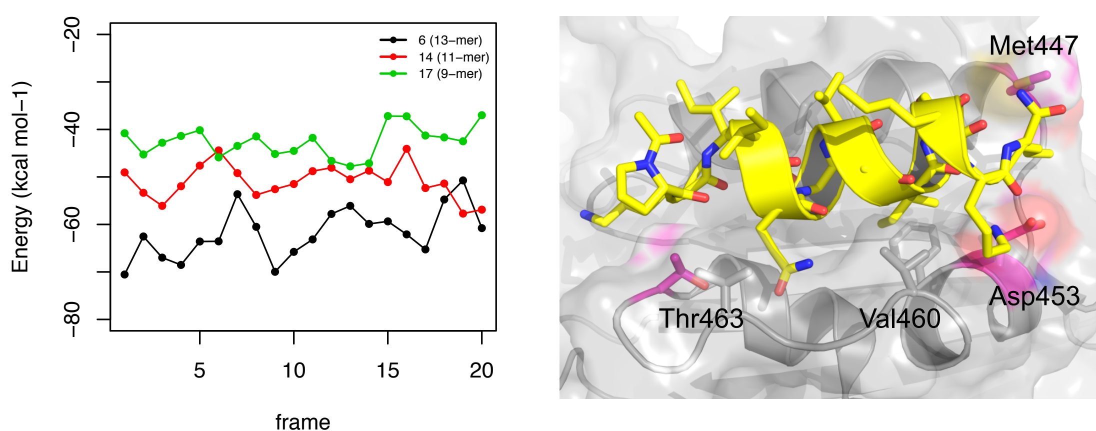


Figure 2. (*Left*) Computed global binding energies (kcal mol^{-1}) for the three representative α/β^3-peptides foldamers **6** (13-mer), **14** (11-mer) and **17** (9-mer). (*Right*) Detail of compound **6** (coloured in yellow) at the *Li*-TryR monomer interface.

We observed that shortening the foldamer (**6** > **14** > **17**) strongly affects the binding affinity for Li-TryR. The loss of the last 4 *C*-terminal residues in **17** (Ser-ß^{3}Nle-Lys-Nle) decreases the binding energy by one third relative to peptide **6** (-61.8 → -42.6 kcal mol^{-1}) and reduce the total number of residues in the protein with a net contribution over 3.0 kcal mol^{-1} for the binding energy (Table 4). A representative example is the strong interaction with Asp453 that is found in **6** (Figure 2) but is lost in the shorter α/β^3-peptides **14** and **17**. In all cases, a significant interaction with Ser440 is found.

Table 4. Binding energy of α/β^3-peptides foldamers **6**, **14** and **17** decomposed by residue (kcal mol^{-1}).

6 (13-mer)			**14** (11-mer)			**17** (9-mer)		
Residue	E	± SD	Residue	E	± SD	Residue	E	± SD
Asp453	-5.5	± 0.6	**Ser440**	-5.2	± 0.4	**Thr457**	-4.4	± 0.7
Thr457	-5.2	± 0.9	**Val460**	-4.8	± 0.6	**Ser440**	-3.7	± 0.4
Val460	-4.6	± 0.5	**Thr463**	-3.1	± 0.5	**Glu436**	-3.5	± 1.5
Cys444	-4.3	± 0.5	**Cys444**	-3.0	± 0.6	**Phe454**	-3.5	± 0.2
Met447	-3.5	± 0.9						
Ser440	-3.4	± 0.5						
Thr463	-3.1	± 0.5						
Phe454	-3.1	± 0.4						
Global interaction energies								
-61.8 ± 5.4			-51.0 ± 3.7			-42.6 ± 3.2		

CONCLUSIONS

Maintaining the recognition properties of prototype peptides while increasing proteolytic stability has been of particular interest for a long time. We herein describe how this goal was achieved with oligomers displaying a specific conformational propensity (foldamers) that contain both α and β^3-amino acid residues (mixed α/β-peptides) designed to target the dimer interface of Li-TryR. A small library of 14 hybrid α/β-peptides of different length and α→β substitution patterns was prepared and evaluated for Li-TryR inhibition of both activity and dimerization. We demonstrated that the 13-residue α/β-peptides were potent inhibitors of both Li-TryR oxidoreductase activity and dimerization while shorter analogues displayed a markedly decreased potency in both assays. The influence of length on the ability of α/β-peptides to disrupt Li-TryR dimerization was rationalized in terms of differences in calculated binding energies with the Li-TryR monomer. Interestingly, several α/β-peptides display significant increases in proteolytic stability against non-specific proteinase K compared to both previous cyclic peptide analogues and the native parent linear α-peptide sequence from which they are derived. These findings highlight the usefulness of the backbone-modified peptide strategy involving periodic α-to-β replacements for designing PPI inhibitors of Li-TryR with enhanced proteolytic stability. Moreover, the most stable mixed α/β-peptide **8**, when conjugated to oligoarginines (compound **20**), exhibits potent activity against *L. infantum* promastigotes and amastigotes. This conjugate displays not only increased potency as a leishmanicidal agent but also decreased toxicity against THP-1 cells in comparison to previous α-peptide R_9-conjugates.

EXPERIMENTAL SECTION

1. Chemistry

1.1. General Methods

Unless otherwise noted, analytical grade solvents and commercially available reagents were used without further purification. DIEA, piperidine, Ac_2O, and EDT were purchased from Aldrich (Germany), TFA from Fluka (Germany) and *O*-(6-chlorobenzotriazol-1-yl)-*1,1,3,3*-tetramethyluronium hexafluorophosphate (HCTU) from Fluorochem (UK). Fmoc-protected Rink Amide MBHA resin (0.56 mmol/g loading) was purchased from *Iris Biotech* (Germany). Fmoc-α-protected amino acids were purchased from Fluka (Germany), Novabiochem (Merck, Germany) and Iris Biotech (Germany). Fmoc-protected β^3-homoamino acids were purchased from AnandChem (Slovak Republic).

α,β^3-peptide foldamers **5-20** were synthesized using the standard Fmoc/^{t}Bu solid-phase orthogonal protection strategy. Compounds were synthesized manually on a 20-positions vacuum manifold (*Omega*) connected to a vacuum pump using 20-mL polypropylene plastic syringes (*Dubelcco*) with a preinserted frit and a Teflon stopcock to do the washings and remove the solvents and excess of the reagents. The coupling reactions were carried out on solid phase using microwave radiation in a *Biotage Initiator* reactor in a 10-mL vial. Excluding the coupling reaction on the microwave reactor, the rest of the SPPS reactions were stirred using an *IKA-100* orbital shaker. The monitoring of the reactions was also performed by HPLC/MS through a HPLC-waters 12695 connected to a Waters Micromass ZQ spectrometer. After cleavage, the acidic crudes were sedimented in Et_2O on a *Hettlich Universal* 320R centrifuge at 5000 rpm. All the crude and samples were lyophilized using mixtures water/acetonitrile on a Telstar 6-80 instrument. The compounds were purified on a SP1 Isolera Biotage instrument using reverse phase columns or on a semipreparative HPLC Waters equipment as detailed below. As mobile phase, mixtures of A:B were used, where A =

0.05% TFA water and B = acetonitrile with a flow rate of 7 mL/min. The peptides were purified using a gradient from 0% of B to 100% of B in 30-45 min and were detected at 217 nm. After purification, α,β^3-peptide foldamers were lyophilized and dried under reduced pressure in the presence of P_2O_5.

The purity of the final products was checked by analytical RP-HPLC on an Agilent Infinity instrument equipped with a Diode Array and a C18 Sunfire column (4.6 mm x 150 mm, 3.5 μm). As mobile phase, A:B mixtures were used, where A = 0.05% TFA water and B = acetonitrile. The samples were analyzed at 214 and 254 nm in a gradient from 2% of B to 100% of B in 15 min (gradient 1). HRMS (EI+) was carried out in an Agilent 6520 Accurate-Mass Q-TOF LC/MS spectrometer using water/acetonitrile. MALDI-TOF mass spectrometer (Voyager DE-STR Applied Biosystems) operated in reflectron mode to obtain better resolution was used for the $[R_9]$-$[\alpha/\beta^3$-peptide] conjugate **20**. Thus, the purity and identity of α,β-peptide foldamers and conjugates was confirmed by HRMS or MALDI-TOF-MS.

General elongation procedure. Fmoc-protected Rink Amide MBHA resin was swollen in DCM/DMF/DCM/DMF (4 x 0.5 min). Then, the resin was treated with 20% piperidine in DMF or with a mixture of DBU:piperidine:DMF (1:1:48, in volume) at room temperature (1 x 1 min) and (3 x 10 min) and washed with DMF/DCM/DMF/DCM (4 x 0.5 min). Later, to the free $N\alpha$-terminal swollen resin (1 equiv), a solution of the corresponding fmoc-α-amino acid or fmoc-β^3-amino acids (1.2 equiv), HCTU (1.2 equiv) and DIEA (2.4 equiv) in DMF (5 mL) was added. After sealing the vial, the reaction was heated in a microwave vial equipped with a magnetic stirrer for 10 minutes at 40 ºC. Then, the vial was opened, the supernatant removed and new coupling mixture added. This process was repeated 3 times in total (3 x 10 min) until complete coupling. Finally, the resin was transferred to a fritted syringe, drained and washed in vacuo extensively (DMF/DCM/DMF/DCM, 5 x 0.5 min). This protocol was repeated for each amino acid. Coupling reactions to primary amines were

monitored by the Kaiser ninhydrin test and to secondary amines by the Choranil test. In some cases, the progress of the reactions was also followed by analysis of a small sample of peptidyl-resin after acidic cleavage in an HPLC-MS instrument.

General acetylation procedure. After elongation of the α,β-peptide foldamers, the N-terminal group was acetylated in all the cases by deprotection of the Fmoc-resin-bounded derivative as mentioned above followed by treatment of the deprotected resin with a mixture of Ac_2O:DIEA:DMF (1:1:1, in volume) at room temperature (1 x 1 min) and (4 x 10 min). The resin was finally washed with DMF/DCM/DMF/DCM (4 x 0.5 min).

General cleavage procedure. The well dried resin-bound derivative (1 volume) in a fritted syringe was treated with TFA:TIPS:H_2O 95:2.5:2.5 (5 volumes) for 4 h at room temperature. In the case of peptides. The filtrates were precipitated over cold Et_2O and centrifuged three times at 5000 rpm for 10 min. After removing the supernatant, the pellet was redissolved in water/acetonitrile and lyophilized.

Purification of α,β-peptide foldamers. The crudes were purified by two different methods to obtain the target α,β-peptide foldamers in high purity:

1) In a SP1 Isolera Biotage equipment using presoaked cartridges KP-C18-HS 12 g (21 x 55 mm). As mobile phase, mixtures of A:B were used, where A = 0.05% TFA water and B = acetonitrile with a gradient of 10 to 20% of acetonitrile in water, in 30 min, with a flow of 12 mL/min. Samples were loaded dissolved or suspended in the minimal quantity of water.

2) In a preparative reverse phase HPLC Waters equipment connected to a Fraction Colector III, using a C18 ACE 5 C18-300 (250 x 10 mm) column. The gradient mobile phases consisted on acetonitrile and water with 0.1% of formic acid as modifier and the gradient consisted of 2 to 95% of strong solvent in 30 min with a flow of 6 mL/min. Samples were loaded dissolved in the minimal quantity of water/acetonitrile/DMSO. The peptides were detected at 217 nm.

1.2. Solid-Phase Peptide Synthesis of α,β-peptide foldamers.

Ac-Pro-Lys- β^3***Ile-Ile-Gln-Ser-*** β^3***Val-Gly-Ile-*** β^3***Ser-Nle-Lys-Nle-NH*** $_2$ (**5**)

Following the general coupling procedure for the synthesis of α,β-peptide foldamers on solid phase, starting with 0.14 mmol of Rink Amide MBHA resin (0.56 mmol/g loading), and prior acetylation and acidic cleavage, the resulting residue was purified in a Isolera Biotage® and lyophilized to obtain **5** as a white solid in a 13% yield. **HPLC**: t_R = 8.62 min (G2-100, 15 min), 95% purity (at 214 nm). **HRMS** (ESI positive) *m/z:* Calculated $C_{70}H_{127}N_{17}O_{17}$ 1477.9596; Found $[M+H]^+$ 1477.9604 (0.58 ppm).

Ac-Pro-Lys-Ile- β^3**Ile-Gln-Ser-** β^3**Val-Gly-Ile-Ser-** β^3**Nle-Lys-Nle-NH** $_2$ **(6)**

The general protocol was followed with 0.14 mmol of resin. After purification of the crude, **6** was isolated as a white lyophilized cotton-like solid (48.05 mg, 23% overall yield). **HPLC**: t_R = 8.99 min (G2-100, 15 min), 91% purity (at 214 nm). **HRMS** (ESI positive) *m/z:* Calculated $C_{70}H_{127}N_{17}O_{17}$ 1477.9596; Found $[M+H]^+$ 1477.9612 (0.27 ppm).

Ac- β^3**Pro-Lys-Ile-** β^3**Ile-Gln-Ser-Val-** β^3**Ala-Ile-Ser-** β^3**Nle-Lys-Nle-NH** $_2$ **(7)**

The general protocol was followed with 0.14 mmol of resin. After purification of the crude peptide, **7** was isolated as a white lyophilized cotton-like solid (45.21 mg, 21% overall yield). **HPLC**: t_R = 9.61 min (G2-100, 15 min), 95% purity (at 214 nm). **HRMS** (ESI positive) *m/z:* Calculated $C_{72}H_{131}N_{17}O_{17}$ 1505.9909; Found $[M+H]^+$ 1505.9903 (-0.39 ppm).

Ac-Pro-Lys- β^3**Ile-Ile-Gln-Gln-** β^3**Ser-Val-Gly-Ile-** β^3**Ser-Nle-Lys-** β^3**Nle-NH** $_2$ **(8)**

Starting from 0.14 mmol of resin, and after following the general protocol, **8** was purified and isolated as a white lyophilized cotton-like solid (53.74 mg, 26% overall yield). **HPLC**: t_R = 8.58 min (G2-100, 15 min), 98% purity (at 214 nm). **HRMS** (ESI positive) *m/z:* Calculated $C_{71}H_{129}N_{17}O_{17}$ 1491.9752; Found $[M+H]^+$ 1491.9721 (-2.09 ppm).

Ac-β^{3}Pro-Lys-Ile-Ile-β^{3}Gln-Ser-Val-β^{3}Ala-Ile-Ser-Nle-β^{3}Lys-Nle-NH$_2$ (9)

The general protocol was followed with 0.112 mmol of resin. The crude peptide was purified to give **9** as a white lyophilized cotton-like solid (37.67 mg, 22% overall yield). **HPLC**: t_R = 8.66 min (G2-100, 15 min), 95% purity (at 214 nm). **HRMS** (ESI positive) *m/z:* Calculated $C_{72}H_{131}N_{17}O_{17}$ 1505.9909; Found $[M+H]^+$ 1505.9916 (0.5 ppm).

Ac-Pro-β^{3}Lys-Ile-Ile-β^{3}Gln-Ser-Val-Gly-β^{3}Ile-Ser-Nle-β^{3}Lys-Nle-NH$_2$ (10)

Starting from 0.112 mmol of resin, and after following the general protocol, **10** was purified and isolated as a white lyophilized cotton-like solid (76.14 mg, 45% overall yield). **HPLC**: t_R = 7.72 min (G2-100, 15 min), 99% purity (at 214 nm). **HRMS** (ESI positive) *m/z:* Calculated $C_{71}H_{129}N_{17}O_{17}$ 1491.9752; Found $[M+H]^+$ 1491.9745 (-0.52 ppm).

Ac-Pro-β^{3}Lys-Ile-Ile-Gln-β^{3}Ser-Val-Gly-β^{3}Ile-Ser-Nle-Lys-β^{3}Nle-NH$_2$ (11)

The general protocol was followed starting from 0.089 mmol of resin. The final residue was purified to yield **11** as a white lyophilized cotton-like solid (15.07 mg, 11% overall yield). **HPLC**: t_R = 8.26 min (G2-100, 15 min), 97% purity (at 214 nm). **HRMS** (ESI positive) *m/z:* Calculated $C_{71}H_{129}N_{17}O_{17}$ 1491.9752; Found $[M+H]^+$ 1491.9701 (-3.47 ppm).

Ac-Pro-Lys-β^{3}Ile-Ile-Gln-Ser-β^{3}Val-Gly-Ile-Ser-β^{3}Nle-Lys-Nle-NH$_2$ (12)

The general protocol was followed starting from 0.14 mmol of resin. The final residue was purified to yield **12** as a white lyophilized cotton-like solid (39.93 mg, 19% overall yield). **HPLC**: t_R = 8.90 min (G2-100, 15 min), 97% purity (at 214 nm). **HRMS** (ESI positive) *m/z:* Calculated $C_{70}H_{127}N_{17}O_{17}$ 1477.9596; Found $[M+H]^+$ 1477.9613 (1.18 ppm).

Ac-Pro-Lys-Ile-β^{3}Ile-Gln-Ser-Val-β^{3}Ala-Ile-Ser-Nle-β^{3}Lys-Nle-NH$_2$ (13)

The general protocol was followed with 0.14 mmol of resin. After purification of the crude peptide, **13** was isolated as a white lyophilized cotton-like solid (47.77 mg, 23% overall

yield). **HPLC**: t_R = 9.20 min (G2-100, 15 min), 97% purity (at 214 nm). **HRMS** (ESI positive) *m/z:* Calculated $C_{71}H_{129}N_{17}O_{17}$ 1491.9752; Found $[M+H]^+$ 1491.9821 (4.58 ppm).

Ac-Pro-Lys-Ile-β ^{3}Ile-Gln-Ser-β ^{3}Val-Gly-Ile-Ser-β ^{3}Nle-NH$_2$ (14)

Starting from 0.112 mmol of resin, and after following the general protocol, **14** was purified and isolated as a white lyophilized cotton-like solid (21.80 mg, 15% overall yield). **HPLC**: t_R = 9.00 min (G2-100, 15 min), 98% purity (at 214 nm). **HRMS** (ESI positive) *m/z:* Calculated $C_{58}H_{104}N_{14}O_{15}$ 1236.7806; Found $[M+H]^+$ 1236.7806 (0.07 ppm).

Ac-Pro-Lys-β ^{3}Ile-Ile-Gln-β ^{3}Ser-Val-Gly-Ile-NH$_2$ (15)

The general protocol was followed with 0.168 mmol of resin. After purification of the crude peptide, **15** was isolated as a white lyophilized cotton-like solid (81.19 mg, 44% overall yield). **HPLC**: t_R = 7.65 min (G2-100, 15 min), 96% purity (at 214 nm). **HRMS** (ESI positive) *m/z:* Calculated $C_{48}H_{86}N_{12}O_{12}$ 1022.6488; Found $[M+H]^+$ 1022.6499 (1.02 ppm).

Ac-Pro-Lys-β ^{3}Ile-Ile-Gln-Ser-β ^{3}Val-Gly-Ile-NH$_2$ (16)

The general protocol was followed with 0.14 mmol of resin. After purification of the crude peptide, **16** was isolated as a white lyophilized cotton-like solid (70.61 mg, 49% overall yield). **HPLC**: t_R = 8.01 min (G2-100, 15 min), 98% purity (at 214 nm). **HRMS** (ESI positive) *m/z:* Calculated $C_{48}H_{86}N_{12}O_{12}$ 1022.6488; Found $[M+H]^+$ 1022.6490 (0.22 ppm).

Ac-Pro-Lys-Ile-β ^{3}Ile-Gln-Ser-β ^{3}Val-Gly-Ile-NH$_2$ (17)

Starting from 0.14 mmol of resin, and after following the general protocol, **17** was purified and isolated as a white lyophilized cotton-like solid (55.26 mg, 39% overall yield). **HPLC**: t_R = 8.10 min (G2-100, 15 min), 98% purity (at 214 nm). **HRMS** (ESI positive) *m/z:* Calculated $C_{48}H_{86}N_{12}O_{12}$ 1022.6488; Found $[M+H]^+$ 1022.6516 (2.75 ppm).

Ac-β^3Pro-Lys-Ile-β^3Ile-Gln-Ser-Val-β^3Ala-Ile-NH_2 (18)

The general protocol was followed with 0.14 mmol of resin. After purification of the crude peptide, **18** was isolated as a white lyophilized cotton-like solid (53.74 mg, 26% overall yield). **HPLC**: t_R = 8.50 min (G2-100, 15 min), 99% purity (at 214 nm). **HRMS** (ESI positive) *m/z:* Calculated $C_{50}H_{90}N_{12}O_{12}$ 1050.6801; Found $[M+H]^+$ 1050.6805 (0.4 ppm).

FITC-PEG-Pro-Lys-β^3Ile-Ile-Gln-β^3Ser-Val-Gly-Ile-β^3Ser-Nle-Lys-β^3Nle-NH_2 (19)

After elongation of the foldamer starting from 0.14 mmol of Rink amide resin, the treatment with PEG and FITC was carried out following the general protocol at room temperature. The crude was purified by semipreparative HPLC to yield the final product **19** as a yellow solid (12.37 mg, 14% overall yield). **HPLC**: t_R = 9.24 min (G2-100, 15 min), 92% purity (at 254 nm). **HRMS** (ESI positive) *m/z:* Calculated $C_{96}H_{149}N_{19}O_{24}S$ 1984.0744; Found $[M+H]^+$ 1984.0754 (0.54 ppm).

Ac-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Pro-Lys-β^3Ile-Ile-Gln-β^3Ser-Val-Gly-Ile-β^3Ser-Nle-Lys-β^3Nle-NH_2 (20)

The general elongation protocol was followed with 0.168 mmol of resin. After purification of the crude, **20** was isolated as a white lyophilized cotton-like solid (36.00 mg, 7% overall yield). **HPLC**: t_R = 4.23 min (G2-100, 15 min), 91% purity (at 214 nm). **MALDI-TOF-MS** $C_{125}H_{237}N_{53}O_{26}$ 2899.08.

The analytical data and overall yields of target α,β-peptide foldamers are detailed in **Table S1** (see Supplementary material).

2. Protease susceptibility assays. Stock solutions of each α/β-peptide foldamer were prepared in tris-buffered saline (TBS buffer, pH = 7.6, Aldrich) with 10% DMSO (for solubility) at 100 μM. A 10 μg/mL stock solution of proteinase K (based on weight to volumen) was prepared in TBS. For each proteolysis reaction, the former foldamer stock

solutions (250 μL) were mixed with TBS (208 μL). Then, proteinase K stock solution (42 μL) was added (final concentration enzyme 10 μg/mL), the solution was mixed, and the reaction was allowed to proceed at room temperatura with orbital shaking. The reaction was quenched at the desired time point (0, 5, 15, 30, 60, 180, 300 and 360 min for α/β-peptides **7** and **12**; 0, 30, 60, 120, 180, 300, 420, 540 min and 24 and 32 h for α/β-peptides **6**, **8**, **10** and **11**; and 0, 5, 10, 30, 60, 120, 180, 240, 300, 360, 480 min and 10, 27 and 48 h for α/β-peptide **15**) by addition of 1% TFA in water (100 μL). 100 μL of the resulting quenched reaction was injected onto an analytical reverse phase HPLC, and the amount of starting foldamer present quantified by integration of the peak at 214 nm. Duplicate or triplicate reactions were run for each time point and half-lives determined by fitting time dependent peptide concentration to an exponential decay using GraphPad Prism.

3. *Li*-TryR oxidoreductase activity. Oxidoreductase activity was determined according to the method described by Hamilton et al. [23] Briefly, reactions were carried out at 26 ºC (250 µL) of HEPES pH 8.0 (40 mM) buffer containing EDTA (1 mM), NADPH (150 µM), NADP+ (30 µM), DTNB (25 µM), T[S]2 (1 µM), glicerol (0.02%), DMSO (1.5%) and recombinant Li-TryR (7 nM). For IC_{50} determinations (**5-18**) the enzyme was pre-incubated with the peptides (concentrations ranging from 75 µM to 0.29 µM) for 10 min prior the addition of $T[S]_2$ and NADPH. Enzyme activity was monitored by the increase in absorbance at 412 nm for 1 h at 26 °C in a VERSAmax microplate reader (Molecular Devices, California, USA). All the assays were conducted in triplicate in at least three independent experiments. Data were analyzed using a nonlineal regression model with the Grafit6 software (Erithacus, Horley, Surrey, UK).

4. Dimer quantitation assay. The stability of the Li-TryR dimeric form in the presence of α/β-peptide foldamers **5-18** was evaluated using the novel Enzyme-Linked ImmunoSorbent Assay (ELISA) recently developed in our laboratory.[9] Briefly a dual (HIS/FLAG) tagged Li-

TryR (400 nM) was incubated in a dimerization buffer (200 μL 300 mM NaCl, 50 mM Tris pH 8.0) for 16 h at 37 °C with agitation and in a humid atmosphere in the presence of different peptide concentration (10 to 90 μM). Next the plates were washed ten times with TTBS (Tween 0.1%, 2 mM Tris, 138 mM NaCl 138 pH 7.6) and incubated with diluted monoclonal α-HIS HRP conjugated antibody (200 μL, Abcam, Cambridge, UK) in BSA (5%) in TTBS for 1 h at room temperature. The plates were washed once again as previously described and 1,2-phenylenediamine dihydrochloride (OPD) substrate (100 μL, Dako, Glostrup, Denmark) prepared according to manufacturer's instructions was added. The enzymatic reaction was stopped after 10 min with H_2SO_4 (100 μL, 0.5 M) and the absorbances were measured at 490 nm in a VERSAmax microplate reader (Molecular Devices, California, USA). All the assays were conducted in triplicate in at least three independent experiments. Data were analyzed using a non-linear regression model with the Grafit6 software (Erithacus, Horley, Surrey, UK).

5. Cells and culture conditions. *L. infantum* axenic amastigotes were grown in M199 (Invitrogen, Leiden, The Netherlands) medium supplemented with 10% heat inactivated FCS, 1 g/L β-alanine, 100 mg/L L-asparagine, 200 mg/L sucrose, 50 mg/L sodium pyruvate, 320 mg/L malic acid, 40 mg/L fumaric acid, 70 mg/L succinic acid, 200 mg/L α-ketoglutaric acid, 300 mg/L citric acid, 1.1 g/L sodium bicarbonate, 5 g/L MES, 0.4 mg/L hemin, 10 mg/L gentamicine pH 5.4 at 37 ºC. THP-1 cells were grown in RPMI-1640 medium (Gibco, Leiden, The Netherlands) supplemented with 10% heat inactivated FCS, antibiotics, 1 mM HEPES, 2 mM glutamine and 1 mM sodium pyruvate, pH 7.2 at 37 ºC and 5% CO_2.

L. infantum promastigotes (MCAN/ES/ 89/IPZ229/1/89) were grown in RPMI-1640 medium (Sigma–Aldrich, St. Louis, MO, USA) supplemented with 10% heat-inactivated fetal calf serum (FCS), antibiotics, and 25 mm HEPES (pH 7.2) at 26 ºC.

6. Leishmanicidal activity and cytotoxicity assays. Drug treatment of amastigotes was performed during the logarithmic growth phase at a concentration of 1×10^6 parasites/mL at 37 ºC for 24. Drug treatment of promastigotes was performed during the logarithmic growth phase at a concentration of 2×10^6 parasites/mL at 26 ºC for 24 h. Drug treatment of THP-1 cells was performed during the logarithmic growth phase at a concentration of 4×10^5 cells/mL at 37 ºC and 5% CO_2 for 24 h. LC_{50} was evaluated by flow cytometry by the propidium iodide (PI) exclusion method [24]. After selection of the parasite population based on their forward scatter (FSC) and side scatter (SSC) values, live and dead parasite cells were identified by their permeability to PI. This is a conservative procedure that may underestimate LC_{50} values as parasites that became fragmented as a consequence of cell death are excluded from the analysis. To minimize the presence of fragmented parasites drug treatment never exceeded 24 h.

7. Molecular modeling. The crystallographic coordinates of *Leishmania infantum* TryR were retrieved from the Protein Data Bank (entry id. 2JK6) [25]. The non-natural β^3-amino derivatives of isoulecine, norleucine and valine were parametrized for the force field using the RED RESP server [26]. The ff14SB force field parameter set in AMBER 16 was used [27]. Each complex foldamer: *Li*-TryR was immersed in a box of 27,000 TIP3P water molecules that extended 12 Å away from any solute atom and Na^+ ions were added to ensure electrical neutrality. The MD simulation protocol at 300 K and 1 atm (NPT ensemble) was carried out as described before [28] using the pmemd_cuda. SPFP module in AMBER16. In this case, the positions of all the Cα atoms of protein residues were restrained (5 kcal mol^{-1}). The energy analysis was carried out using our in-house program MM-ISMSA [29] on 20 snapshots taken from the last 10 ns of the simulation that were previously cooled down to 100 K over 1 ns of simulation during which the former positional constraints were kept.

Appendix A. Supplementary data

The analytical data and overall yields of final α,β,-peptides **5-20** (Table S1), HPLC, HRMS or MALDI-TOF-MS of α,β,-peptide foldamers analogues, and proteolysis stability data are also included.

Supplementary data related to this article can be found at

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REFERENCES

1) www.who.int/mediacentre/factsheets/fs375/en updated april 2017.

2) a) M.C. Field, D. Horn, A.H. Fairlamb, M.A.J. Ferguson, D.W.Gray, K.D. Read, M. De Rycker, L.S. Torrie, P.G. Wyatt, S.Wyllie, I.H. Gilbert, Anti-trypanosomatid drug discovery: an ongoing challenge and a continuing need, Nature Reviews 15 (2017) 217-231, b) Joo Hwan No, Visceral leishmaniasis: revisiting current treatments and approaches for future discoveries. Acta Tropica (2016) 113-123.

3) a) I. Silva-Jardim, O.H. Thiemann and F.F. Anibal, Leishmaniasis and Chagas Disease Chemotherapy: a Critical Review, J. Braz. Chem. Soc. 25 (2014) 1810-1823, b) A.S. Nagle, S. Zhare, A.B. Kumar, F. Supek, A. Buchynskyy, C.J.N. Mathison, N.K. Chennamaneni, N. Pendem, F.S. Buckner, M.H. Gelb, V. Molteni, Recent developments in drug discovery for Leishmaniasis and Human African Trypanomiasis, Chem. Rev. 114 (2014) 11305-11347, c) M. Yasinzai, M. Khan, A. Nadhman, G. Shahnaz, Drug resistance

in leishmaniasis: current drug-delivery systems and future perspectives, Fut. Med. Chem. 5 (2013) 1877-1888.

4) A.H. Fairlamb, P. Blackburn, P. Ulrich, B.T. Chait and A. Cerami, Trypanothione: a novel bis(glutathionyl)spermidine cofactor for glutathione reductase in trypanosomatids, Science 227 (1985) 1485-1487.

5) R.L. Krauth-Siegel, H. Bauer and R.H. Schirmer, Dithiol proteins as guardians of the intracelular redox milieu in parasites: old and new drug targets in trypanosomes and malaria-causing plasmodia, Angew. Chem. Int. Chem. 44 (2005) 690-715.

6) See for example, L.S.C. Bernades, C.L. Zani and I. Carvalho, Trypanosomatidae Diseases: From the Current Therapy to the Efficacious Role of Trypanothione Reductase in Drug Discovery, Curr. Med. Chem. 20 (2013) 2673-2696 and references therein.

7) K. Suresh, A. Md Rahmat, B. Sandhya, Mini review on tricyclic compounds as an inhibitor of trypanothione reductase, Journal of pharmacy & bioallied sciences 6 (2014) 222-8.

8) M. Omar, F. Khan, Trypanothione Reductase: A Viable Chemotherapeutic Target for Antitrypanosomal and Antileishmanial Drug Design, Drug Target Insights 2 (2007) 129-146.

9) M.A. Toro, P.A. Sánchez-Murcia, D. Moreno, M. Ruiz-Santaquiteria, J.F. Alzate, A. Negri, M.J. Camarasa, F. Gago, S. Velázquez, and A. Jiménez-Ruiz, Probing the dimerization interface of Leishmania infantum trypanothione reductase with site-directed mutagenesis and short peptides, ChemBioChem 14 (2013) 1212-1217.

10) C. Adessi, C. Soto, Converting a peptide into a drug: strategies to improve stability and bioavailability, Curr Med Chem 9 (2002) 963-978.

11) L. Gentilucci, R. De Marco, L. Cerisoli, Chemical modifications designed to improve peptide stability: incorporation of non-natural amino acids, pseudo-peptide bonds and cyclization, Curr Pharm Des 16 (2010) 3185-3201.

12) See for example recent reviews: a) P. Wojcik, L. Berlicki, Peptide-based inhibitors of protein-protein interactions, Bioor. Med. Chem. Lett. 26 (2016) 707-713, b) P.M. Cromm, J. Spiegel, T.N. Grossmann, Structure-based design of inhibitors of protein-protein interactions: mimicking peptide binding epitopes, Angew. Chem. Int. Ed. 54 (2015) 8896-8927.

13) P.A. Sánchez-Murcia, M. Ruiz-Santaquiteria, M.A. Toro, H. De Lucio, M.A. Jiménez, F. Gago, A. Jiménez-Ruiz M.J. Camarasa, S. Velázquez, Comparison of hydrocarbon- and lactam-bridged cyclic peptides as dimerization inhibitors of of Leishmania infantum trypanothione reductase, RSC Adv. 5 (2015) 55784-55794.

14) M. Ruiz-Santaquiteria, P.A. Sánchez-Murcia, M.A. Toro, H. de Lucio, K.J. Gutiérrez,S. de Castro, F.A.C. Carneiro, F. Gago, A. Jiménez-Ruiz, M.J. Camarasa, S. Velázquez, First example of peptides targeting the dimer interface of Leishmania infantum trypanothione reductase with potent in vitro antileishmanial activity, Eur. J. Med. Chem. 135 (2017) 49-59.

15) For example, see recent review: R. Gopalakrishnan, A.I. Frolov, L. Knerr, W.J. Drury, E. Valeur, Therapeutic potential of foldamers: from chemical biology tools to drug candidates?, J. Med. Chem. 59 (2016) 9599-9621.

16) W.S. Horn, M.D. Boersma, M.A. Windsor, S.H. Gellman, Sequence-based design of α/β-peptide foldamers that mimic BH3 domains, Angew. Chem. Int. Ed. 47 (2008) 2853-2856.

17) M.D. Boersma, H.S. Haase, K.J. Peterson-Kaufman, E.F. Lee, O.B. Clarke, P.M. Colman, B.J. Smith, W.S. Horne, W.D. Fairlie, S.H. Gellman, Evaluation of diverse α/β-backbone

patterns for functional α-helix mimicry: analogues of the Bim BH3 domain, J. Am. Chem. Soc. 134 (2012) 315-323.

18) L.M. Johnson, S.H. Gellman, α-helix mimicry with α/β-peptides, Methods Enzymol. 523 (2013) 5407-429.

19) E.F. Lee, B.J. Smith, W.S. Horne, K.N. Mayer, M. Evangelista, P.M. Colman, S.H. Gellman, Structural basis of Bcl-xL recognition by a BH3-mimetic of α/β-peptide generated by sequence-based design, ChemBioChem 12 (2011) 2025-2032.

20) L.M. Johnson, W.S. Horne, S.H. Gellman, Broad distribution of energetically important contacts across an extended protein interface, J. Am. Chem Soc. 133 (2011) 10038-10041.

21) W.S. Horne, L.M. Johnson, T.J. Ketas, P.J. Klasse, M. Lu, J.P. Moore, S.H. Gellman, Structural and biological mimicry of protein surface recognition by α/β-peptide foldamers, Proc. Natl. Acad. Sci. U.S.A. 106 (2009)14751-14756.

22) For recent successful examples of α/β-peptide foldamers see: a) R.W. Cheloha, A. Maeda, T. Dean, T.J. Gardella, S.H. Gellman, Backbone modification of a polypeptide drug alters duration of action in vivo, Nat. Biotechnol. 32 (2014) 653-655, b) L.M. Johnson, S. Barrick, M.V. Keller, A.D. Attie, A. Saghatelian, A. Bisello, S.H. Gellman, A Potent α/β-Peptide Analogue of GLP-1 with Prolonged Action in Vivo, J. Am. Chem. Soc. 136 (2014) 12848-12851, c) R.W. Cheloha, T. Watanabe, T. Dean, S.H. Gellman, T.J. Gardella, Backbone modification of a parathyroid hormone receptor-1 antagonist/inverse agonist, ACS Chem. Biol. 11 (2016) 2752-2762.

23) C.J. Hamilton, A. Saravanamuthu, I.M. Eggleston, A.H. Fairlamb, Ellman's-reagent-mediated regeneration of trypanothione in situ: substrate-economical microplate and time-dependent inhibition assays for trypanothione reductase. Biochem. J. 369 (2003) 529-537.

24) J.F. Alzate, A. Arias, F. Mollinedo, E. Rico, J. de la Iglesia-Vicente, A. Jimenez-Ruiz, Edelfosine induces an apoptotic process in *Leishmania infantum* that is regulated by the

ectopic expression of Bcl-XL and Hrk. Antimicrob. Agents Chemother. 52 (2008) 3779-3792.

25) P. Baiocco, G. Colotti, S. Franceschini, A. Ilari, A. Molecular basis of antimony treatment in Leishmaniasis. J. Med. Chem. 52 (2009) 2603-2612.

26) URL: q4md-forcefieldtools.org/REDServer/

27) URL: http://ambermd.org

28) P.A. Sánchez-Murcia, J.A. Bueren-Calabuig, M. Camacho-Artacho, A. Cortés-Cabrera, F. Gago, Stepwise Simulation of 3,5-Dihydro-5-methylidene-4H-imidazol-4-one (MIO) Biogenesis in Histidine Ammonia-lyase, Biochemistry 55 (2016) 5854-5864.

29) J. Klett, A. Núñez-Salgado, H.G. Dos Santos, A. Cortés-Cabrera, A. Perona, R. Gil-Redondo, D. Abia, F. Gago, A. Morreale, MM-ISMSA: An Ultrafast and Accurate Scoring Function for Protein–Protein Docking, J. Chem. Theory Comput. 8 (2012) 3395-3408.