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D-Proline-based peptidomimetic inhibitors of anthrax lethal factor

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ABSTRACT

In this work we reported the generation of D-proline-derived hydroxamic acids as inhibitors of anthrax lethal factor (LF), taking advantage of a pyrrolidine ring as the central scaffold and a hydroxamate group as the Zn^{2+} chelating agent. The introduction of two hydrophobic groups addressing the S1′ subsite and a long substrate-binding groove was conceived by overlapping the bioactive conformations of two reported LF inhibitors. Micromolar affinity of compound **38** suggested *cis*-3-substituted-1-sulfonamido-D-proline hydroxamic acids as a promising class of peptidomimetic inhibitors for developing novel LF inhibitors.

1. Introduction

Anthrax is a disease caused by *Bacillus anthracis* a Gram positive bacterium usually found in the soil [1,2]. It is an epizootic disease to which most mammals are considered susceptible. This disease can be transmitted to humans through contact with infected animals, and no cases of human-to-human transmission have been reported, so far. Due to the risk of using *B. anthracis* spores as a weapon of bioterrorism against human populations, there has been a growing interest towards the development of a treatment for anthrax during the last decade. The clinical effect of this infectious disease depends on its entry route to the body. While entry of *B. anthracis* spores in the human body through skin causing cutaneous anthrax is easily curable with antibiotics, inhalation of anthrax spores is usually fatal. After inhalation, the spores survive and sprout within macrophages, allowing them to reach lymph nodes, and ultimately to start bacteria proliferation in the circulation system [3–5]. The anthrax toxin [6] consists of three secreted proteins, the protective antigen (PA) the lethal factor (LF) and the oedema factor (EF), which are crucial for killing host macrophages and suppressing the immune system's activity [7–9]. The protective antigen PA is necessary for lethal and oedema factors to enter the cell, and binary combinations of PA with the two proteins LF and EF lead to the formation of oedema toxin and of lethal toxin, respectively [10]. PA is activated proteolitically on the host cell surface by the furin-like proprotein convertase, then the bioactive PA fragment heptamerizes to form a membrane pore, and binds to both LF and EF. This complex is then endocytosed, and lethal and oedema factor are released in the cytosol by a denaturation–renaturation process, where they exert their toxic action. EF is a calmodulin-activated adenylate cyclase which causes an increase of cAMP concentration in the cells [11], and LF, is a zinc-dependent metalloproteinase, that has been recognized as the primary and most important virulence factor of *B. anthracis*. The anthrax lethal factor cleaves several members of the mitogen-activated protein kinase kinase (MAPKK) family near their N-terminus [12–15]. This proteolytic activity prevents phosphorylation of MAPKs, and consequently leads to disruption of many signaling pathways and ultimately to cell-death. Given the crucial role that anthrax LF plays in the infectious process, this enzyme is considered a potential therapeutic target of anthrax. Indeed, a possible therapeutic approach envisages the combination of antibiotics with LF inhibitors to clear the active infection while blocking the toxin already present in the cells. There are a number of successful LF inhibitors reported to date [16–21], and important structure–activity studies for understanding binding mode features of chemically diverse inhibitors have been published during the last decade [22,23]. Also, recent reports on potent hydroxamic acid-based LF inhibitors [24,25], and new SAR studies have been reported [26]. Herein, we describe a rational drug design approach that allowed for the discovery of

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new D-proline-derived peptidomimetic LF inhibitors as a new entry to LF inhibitors, characterized by a pyrrolidine ring as the central scaffold and a hydroxamate group as the Zn^{2+} chelating agent.

2. Results and discussion

2.1. Structure alignment and rational design

Preliminary experiments validated the applicability of arylsulfonyl-D-pyrrolidine hydroxamic acids as LF inhibitors in the micromolar range, and both 4-chloro and 3,4-dichlorobenzenesulfonyl groups were selected as substituents at the nitrogen atom. Nevertheless, unsatisfactory logP values for this set of compounds precluded further consideration as inhibitors for intracellular assays (unpublished data). Thus, we reasoned to develop a new set of D-proline-based inhibitors possessing two hydrophobic groups, in order to allow for better pharmacokinetic properties of the inhibitor, and possibly improving their specific binding activity. Accordingly, we considered the design of second generation inhibitors taking into account the binding mode of two diverse inhibitors known in the literature to enable the design of new peptidomimetics possessing D-proline as the core scaffold. Two well-known anthrax lethal factor inhibitors were chosen as model structures, namely the inhibitor L915 developed by Merck and BI-MFM3 by Cengent laboratories, in order to generate a binding interaction model, which provided key structural features required to design novel anthrax LF inhibitors (Fig. 1) [27,28].

BI-MFM3 is a phenylfuran-2-yl-methylene-rhodanine acetic acid derivative. In this case, crystallographic data displayed the Zn^{2+} metal ion interacting at the rhodanine ring via the sulfur atoms of 2-thioxothiazolidine-4-one moiety. Moreover, the phenyl ring group established interactions with hydrophobic side chains of LF, which belong to a binding pocket different from that addressed by the 4-fluoro-3-methylphenyl substituent of L915. L915 is a competitive hydroxamate LF inhibitor, for which the crystal structure in complex with LF was solved. The oxygen atoms of the hydroxamate moiety chelates the Zn^{2+} ion in a bidentate and planar conformation, and the substituted phenyl ring is located in a deep hydrophobic pocket adjacent to the catalytic center. The tetrahydropyranyl moiety, which is located in a large cavity between two different protein domains, is not crucial for the ligand–protein interaction. These crystallographic data highlighted that the binding affinity of L915 to LF is driven by the interactions between the hydroxamic acid and the metal ion, and between the 4-fluoro-3-methylphenyl substituent and the S1′ pocket. The coordinates of these two complexes were superimposed using Swiss PDB viewer, and then protein superimposition was discarded allowing for retaining the alignment of the inhibitors. From the superimposition of the two inhibitors we obtained

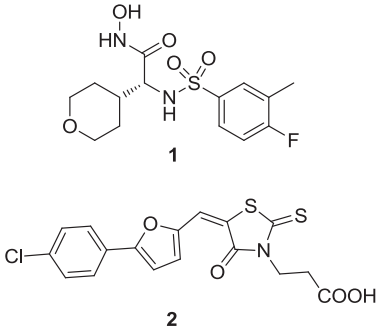


Fig. 1. Merck L915 LF inhibitor (top) and Cengent BI-MFM3 LF inhibitor (bottom).

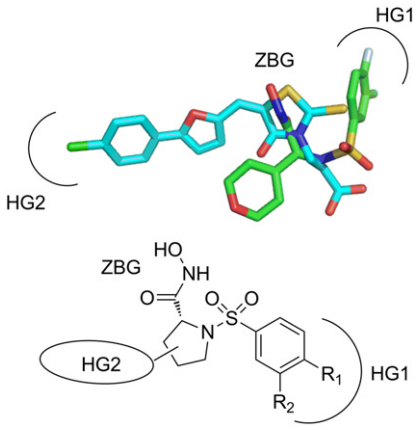


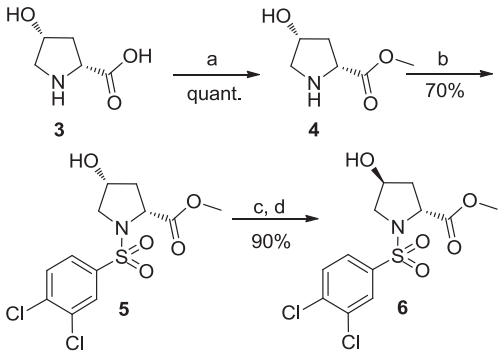
Fig. 2. Superimposition of two reference inhibitors (top) as obtained from the structure alignment of two LF coordinates from X-ray data (PDB codes: 1YQY and 1ZXV), and the resulting designed D-proline-based class of compounds (bottom). ZBG is Zn-Binding Group; HG1 is Hydrophobic Group 1; HG2 is Hydrophobic Group 2.

a pharmacophoric model, which was the starting point for the design of new LF inhibitors (Fig. 2). The binding interaction model obtained by such superimposition is characterized by a central template, a Zn^{2+} chelating agent as represented by the hydroxamate moiety, and by the presence of two hydrophobic groups (HG1 and HG2) addressing two different cavities explored by the reference inhibitors herein taken into account. Taking advantage of this analysis, we considered the pyrrolidine ring of D-proline as the central scaffold, and 4-chloro- or 3,4-dichlorophenylsulfonyl moieties at the nitrogen atom, similarly to L915 inhibitor, as the hydrophobic groups for addressing the deep hydrophobic S1′ subsite (Fig. 2).

Accordingly, we synthesized an array of D-proline hydroxamic acid peptidomimetics containing an additional substituent in order to study the introduction of hydrophobic (HG) groups, as well as the preferred stereochemical arrangement. Initially, we explored the effect of changing substituents and stereochemistry starting from 4-hydroxy-D-proline scaffold.

2.2. Synthesis

Taking into account *cis*-4-hydroxy-D-proline as the chiral template, the key intermediates (2*R*,4*R*) and (2*R*,4*S*)-methyl 1-((3,4-dichlorophenyl)sulfonyl)-4-hydroxypyrrolidine-2-carboxylate were achieved in good yield via the route as shown in Scheme 1. After esterification of *cis*-4-hydroxy-D-proline (**3**) with

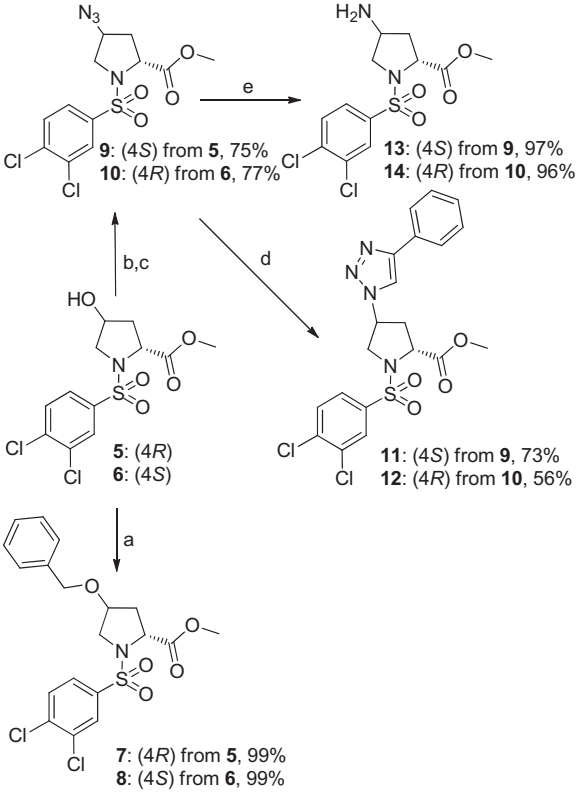


Scheme 1. Reagent and conditions: (a) $SOCl_2$, MeOH, r.t., 16 h; (b) 3,4 dichlorobenzene-1-sulfonyl chloride, Et_3N, DMAP, dry CH_2Cl_2, N_2, r.t., 16 h; (c) $ClCH_2CO_2H$, PPh_3, DIAD, dry toluene; (d) $NaHCO_3$, MeOH.

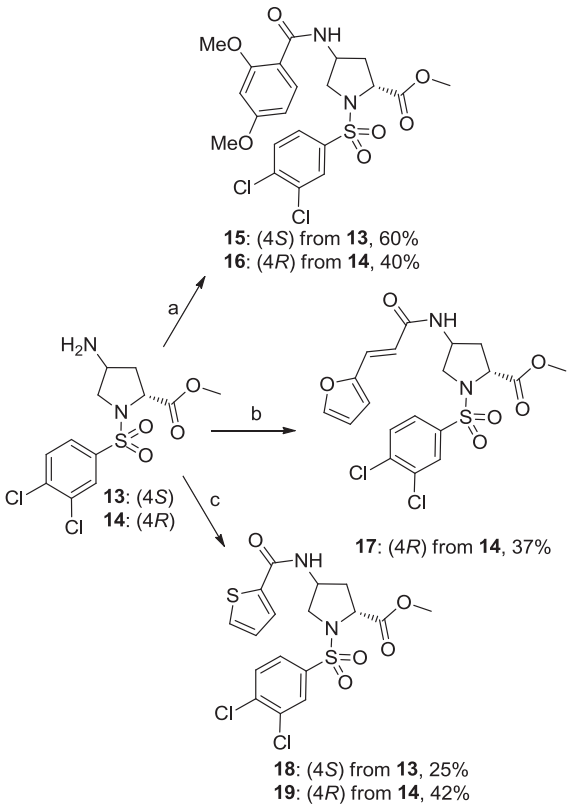
thionyl chloride in methanol to give **4**, and subsequent sulfonamide formation, an inversion of configuration through a Mitsunobu reaction was performed on **5** to obtain the corresponding (2*R*,4*S*)-isomer **6**.

Sulfonamides **5** and **6** were used to prepare the final methyl ester derivatives **7**–**14** through the synthetic pathway as shown in Scheme 2. Benzylation of compound **5** using benzyltrichloroacetimidate afforded the ether **7** in nearly quantitative yield. The corresponding isomer **8** was obtained in the same way starting from compound **6**. Mesylation and subsequent nucleophilic substitution on sulfonamide **5** with NaN_3 produced azide **9** with inverted configuration at the C-4 stereocenter. This compound was combined with ethynylbenzene through click-chemistry to prepare the corresponding methyl ester **11** characterized by a 4-phenyltriazole **11**. Compound **9** was also subjected to catalytic hydrogenation using 10% Pd/C to give amine **13**. Similarly, *trans*-4-hydroxy-proline derivative **6** gave access to *cis*-4-azido-derivative **10**, and consequently the corresponding click-adduct **12** and *cis*-4-aminoproline **14**. Compounds **13**–**14** proved to be a good starting point to introduce amides at position 4 of the pyrrolidine scaffold (Scheme 3). With exception of compound **17** obtained from **14**, 4-aminoproline precursors **13**–**14** were applied for generating a pool 4-amido derivatives as represented by **15**–**19**, in order to have chemical and stereochemical diversity for the second hydrophobic group (HG2), as shown in Fig. 2. The methyl ester of compounds **7**–**8**, **11**–**12** and **15**–**19** was then directly converted to corresponding hydroxamic acids using NH_2OK/NH_2OH in MeOH, as shown in Scheme 4, to produce the corresponding compounds **20**–**28** (Table 1) [29].

In order to modulate the position of HG2 and to study its effect on LF inhibition, we also considered position 3 of the pyrrolidine

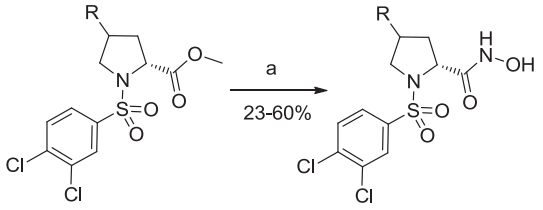


Scheme 2. Representative synthesis of sulfonamides **7**–**14** starting from intermediates **5** and **6**. Reagent and conditions: (a) benzyltrichloroacetimidate, CF_3SO_3H, 3Å MS, Et_2O, r.t., 3 h; (b) MsCl, Et_3N, DCM, r.t., 2 h; (c) NaN_3, DMF, 90 °C, 16 h; (d) ethynylbenzene, $Cu(OAc)_2$, sodium ascorbate, H_2O/*t*-BuOH, r.t., 16 h; (e) 10% Pd/C, H_2, MeOH, r.t., 16 h.



Scheme 3. Reagents and conditions: (a) 2,4-dimethoxybenzoyl chloride, Et_3N, dry CH_2Cl_2, r.t., 16 h; (b) (*Z*)-3-(furan-2-yl)acryloyl chloride, Et_3N, dry CH_2Cl_2, r.t., 16 h; (c) thiophene-2-carbonyl chloride, Et_3N, dry CH_2Cl_2, r.t., 16 h.

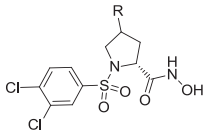
scaffold to install the second hydrophobic group, while retaining the preferred *cis*-configuration (see inhibition data). A relative straightforward route to achieve the desired *cis*-3-hydroxyproline scaffold involved the stereoselective reduction of 3-ketoproline (Scheme 5) [30,31]. *N*-Boc-protected diester **30** was prepared in two steps starting from ethyl glycinate **29** and ethyl acrylate. A Dieckmann cyclization was then conducted to yield racemic β-ketoester **31**. Finally, baker's yeast reduction of the ketone afforded the optically active β-hydroxyester **32**, which was used to obtain final hydroxamic acids **39**–**41** (Scheme 6).

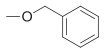
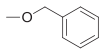
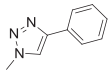
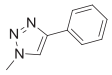
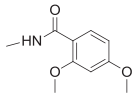
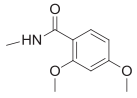
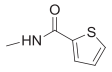
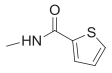
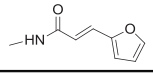


7,**20**: benzyloxy, (4*R*)
8,**21**: benzyloxy, (4*S*)
11,**22**: 4-phenyl-1*H*-1,2,3-triazol-1-yl, (4*S*)
12,**23**: 4-phenyl-1*H*-1,2,3-triazol-1-yl, (4*R*)
15,**24**: 2,4-dimethoxyphenyl-CONH-, (4*S*)
16,**25**: 2,4-dimethoxyphenyl-CONH-, (4*R*)
17,**28**: (furan-2-yl)acrylamido, (4*R*)
18,**26**: thiophene-2-carboxamido, (4*S*)
19,**27**: thiophene-2-carboxamido, (4*R*)

Scheme 4. General procedure for the synthesis of hydroxamic acids. Reagents and conditions: (a) $NH_2OH{\cdot}HCl$, KOH 0.87M in MeOH, r.t., 48–72 h.

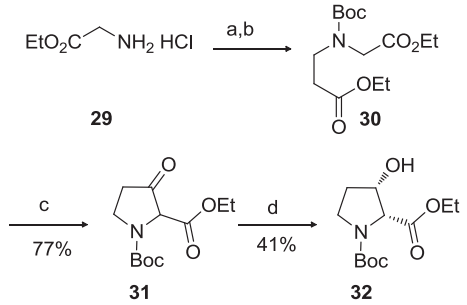
Table 1
Array of 4-hydroxy-proline derivatives assayed towards anthrax lethal factor.



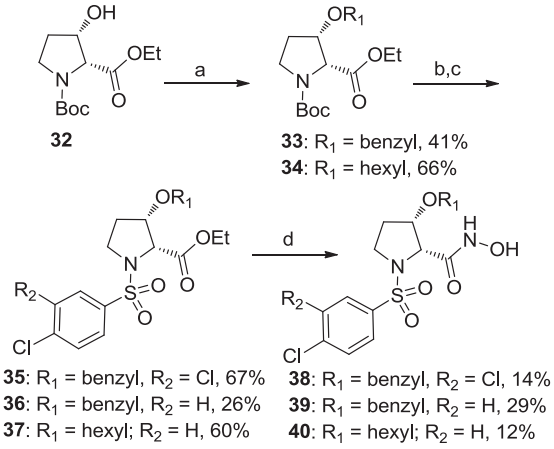
Compound	R	C-4 absolute configuration	% Inhibition[a]
20		*R*	50
21		*S*	16
22		*S*	–
23		*R*	8
24		*S*	11
25		*R*	65
26		*S*	46
27		*R*	27
28		*S*	22

[a] The inhibitory potency was determined using the initial velocity value, which was compared with that in absence of potential inhibitors. Values are reported as the mean of three experiments.

The hydroxylic group at position 3 of the pyrrolidine ring was treated with NaH and benzyl bromide or 1-iodohexane, affording the corresponding ethers **34** and **35**, respectively. After Boc removal, the sulfonamide functionality was introduced as for **5**, and the final hydroxamic acids were prepared by a procedure similar to that followed for 4-hydroxyproline derivatives **20**–**28**.



Scheme 5. Reagents and conditions: (a) ethyl acrylate, Et_3N, EtOH, r.t., 48–72 h; (b) Boc_2O, 5% NaOH, H_2O, r.t., 16 h; (c) *t*-BuOK, toluene dry, N_2, 0 °C, 1.5 h; (d) Baker's yeast, H_2O, 30 °C, 48 h.



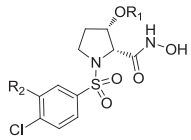
Scheme 6. Reagents and conditions: (a) benzyl bromide, NaH, dry THF, TBAI, N_2, r.t., 16 h for **33**, or 1-iodohexane, NaH, dry DMF, r.t., 16 h for **34**; (b) 1:1 TFA/DCM, r.t., 2–3 h; (c) 3,4 dichlorobenzene-1-sulfonyl chloride, Et_3N, DMAP, dry CH_2Cl_2, N_2, r.t., 16 h for **35**, or 4 chlorobenzene-1-sulfonyl chloride, Et_3N, DMAP, dry CH_2Cl_2, N_2, r.t., 16 h for **36** and **37**; (d) $NH_2OH{\cdot}HCl$, KOH, MeOH, μW, 80 °C, 1h or $NH_2OH{\cdot}HCl$, KOH, MeOH, r.t., 48 h.

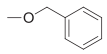
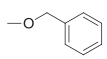
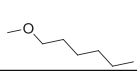
2.3. Enzyme inhibition assay

The ability of this set of peptidomimetics to inhibit anthrax lethal factor activity was evaluated through a spectrophotometric assay using the anthrax lethal factor protease substrate II. Cleavage was measured by monitoring the increase in absorbance at 405 nm corresponding to the release of *p*-nitroaniline. Initially, inhibition studies were carried out at a fixed concentration of potential inhibitors (10 μM), and the percentage of inhibition was calculated (Table 1). The evaluation of the 4-substituted hydroxamic acid-based peptidomimetics as LF inhibitors resulted in the identification of compounds **20** and **25**, exhibiting inhibition potency equal or above 50% at 10 μM concentration. The first round of biological screening on this set of molecules revealed a preference for the *cis*-configuration (4*R* configuration) in giving better inhibition toward the target enzyme. This tendency suggested a role of the absolute configuration at the C-4 stereocenter for inhibition of LF.

IC_{50} values for compounds **20** and **25** were calculated by dose–response measurements using inhibitors in a range of concentrations between 0.5 and 10 μM (Table 2). The two compounds showed moderate inhibitory potency, possessing IC_{50} values in low μM

Table 2
IC_{50} values of 3-hydroxyproline derivatives.



Compound	R_1	R_2	IC_{50} (μM)
38		Cl	1.4
39		H	1.7
40		H	>10

range. Compound **20** displayed low inhibition potency toward LF activity (IC_{50} = 9.5 μM), whereas **25** showed better inhibition capability (IC_{50} = 2.1 μM), suggesting the beneficial effect of including an aromatic amido group at the 4-*cis* position.

In order to study the effect of the position of HG2 towards inhibition, *cis*-3-substituted compounds were assayed for LF inhibition, and IC_{50} values obtained from the screening are shown in Table 2. Compounds **38**–**40** displayed a clear increase in the inhibition potency as compared to the 4-hydroxy-proline analogue **20**. The substituents in that position of the scaffold may fit in the hydrophobic substrate-binding groove better than 4-hydroxy-proline derivatives, by establishing favorable interactions with enzymatic hydrophobic side chains. This result highlighted this substituent arrangement to match our initial hypothesis of binding interaction model better than 4-hydroxyproline derivatives. On the other hand, compound **40** showed a complete loss of potency toward LF, indicating a detrimental effect of the linear alkyl substituent in the inhibition mechanism. The lack of efficacy could be caused by flexibility of this substituent, which can assume an unfavorable conformation in LF binding groove or by the absence of the aromatic ring which may have a specific role in the interaction with the enzyme.

2.4. Molecular modeling

Docking studies were carried out with active compound **38** and the crystal structure of anthrax LF (PDB code: 1YQY) to give more insight into the possible binding mode of this D-proline-based compound to the target enzyme. GOLD software was used to assess the interactions established between the peptidomimetic and the LF active site. Docking results of compound **38** showed a main cluster of conformations characterized by the typical binding mode of hydroxamate-based LF inhibitors (Fig. 3). Specifically, in agreement with the crystallographic structure of LF-inhibitor L915 obtained by Merck, compound **38** showed the oxygen atoms of the hydroxamic acid moiety chelating the catalytic zinc ion.

The aromatic substituents addressed both the hydrophobic substrate-binding groove and the S1′ subsite adjacent to the catalytic Zn^{2+} binding site. In particular, the phenylsulfonyl moiety was located in the S1′ cavity, whereas the benzyl group was oriented in the long substrate-binding groove. These results are particularly attractive because, as shown elsewhere [22,23], this long recognition groove and the deep S1′ pocket are the main determinants for tight-binding inhibition.

2.5. Structure–activity relationship

Taking advantage of the structural informations obtained from the superimposition of the bioactive conformations of two reference inhibitors, we developed an array of peptidomimetics possessing the key hydroxamate group as the Zn^{2+} chelating agent and a pyrrolidine ring as the central scaffold. The introduction of two hydrophobic groups addressing the S1′ subsite and a long substrate-binding groove was explored in the proline scaffold in order to access an improved lethal factor inhibitor. As regarding to the S1′ subsite, both 4-chloro and 3,4-dichlorobenzenesulfonyl groups were selected as substituents at the nitrogen atom. The application of both benzyl or benzoyl residues at the nitrogen atom of the scaffold proved to impair the inhibitory activity (data not shown), thus suggesting a key role of the sulfonamide in addressing the catalytic site. This hypothesis was corroborated by molecular docking calculation, as a hydrogen-bond between an oxygen atom of this group and Gly656 amide proton was observed (Fig. 3).

As regarding to the second hydrophobic group (HG2), an array of the position on the scaffold and the stereochemistry was taken into account to introduce hydrophobic substituents with some chemical diversity between them. Benzyloxy derivative **20** and 2,4-dimethoxybenzamido compound **25**, both possessing the (R) configuration, showed best inhibition profile for 4-substituted proline derivatives (Table 1). Highly constrained aromatic groups for HG2, as represented by phenyltriazolyl derivatives **22** and **23** proved to impair the inhibition profile in either stereochemical orientation, suggesting a stringent topological requirement to address the corresponding region of the catalytic site identified as the long substrate-binding groove. Finally, differences in the type of amido substituent at this position were found to be not significant,

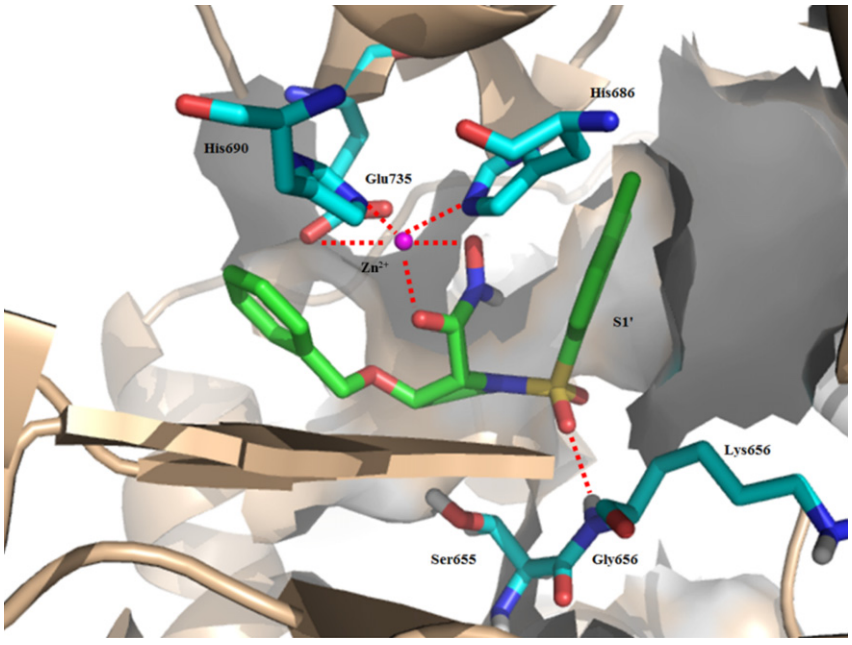


Fig. 3. Best-scoring docked conformations resulting from the docking calculation of compound **38**.

as observed by similar IC50 values of thienyl derivatives as compared to 2,4-dimethoxybenzamido compounds (Table 1).

The improved inhibition of compounds **38** and **39**, bearing a benzyloxy group at position *cis*-3, with respect to the corresponding isomer **20**, possessing the same group at position 4 with similar stereochemistry, suggested that an HG2 at position 3 of the scaffold may fit in the hydrophobic substrate-binding groove better than 4-substituted-proline derivatives. Moreover, the complete loss of potency toward LF observed for compound **40** suggest a specific role of aromatics as HG2 in the interaction with the enzyme, as compared to inhibitory activity of compounds **38** and **39**.

Thus, all these informations suggest that the proline scaffold may be a feasible peptidomimetic scaffold to achieve a lethal factor inhibitor with improved performance. The HG1 consisting of a substituted benzenesulfonyl group may be further explored taking into account more interactive moieties such as polar groups like guanidine-isosteres or hydroxyls. As regarding to HG2, the position 3 was established as being the most promising with a *cis* geometry with respect to HG1. In this view, an array of heteroaromatic group possessing more interactive moieties for the interaction within the long substrate-binding groove, and an overall hindrance similar to the benzyloxy-derivative **38** may give access to an improved inhibitor.

3. Conclusion

In conclusion, we reported the design, synthesis and enzyme inhibition assay of an array of D-proline-derived peptidomimetic hydroxamic acids possessing an aromatic sulfonyl group at the nitrogen atom and another hydrophobic group at position 3 or 4 of the pyrrolidine ring. The structure–activity analysis of this class of compounds showed the importance of the *cis*-configuration of the hydrophobic substituent with respect to the hydroxamic acid, and improved activity when the HG2 was found at position 3 of the pyrrolidine ring. Docking calculations confirmed the HG2 at position 3 to adapt in the long substrate-binding groove, thus enabling future hit-to-lead processes on this class of peptidomimetic LF inhibitors taking into account this molecular geometry.

4. Experimental section

4.1. General

Chromatographic separations were performed on silica gel (Kieselgel 60, Merck) using flash-column techniques or automatic Biotage Isolera system; R_f values refer to TLC carried out on 25-mm silica gel plates (Merck F_{254}) with the same eluant as indicated for column cromatography. ^{1}H NMR spectra were recorded with Varian Gemini and Inova NMR spectrometers operating at 200, 300 and 400 MHz for the proton and 50 and 100 MHz for the carbon, respectively. ESI mass spectra were carried out on an ion-trap double quadrupole mass spectrometer using electrospray (ES^+) ionization techniques, and a normalized collision energy within the range of 21–28 eV for MSMS experiments. All the compounds were found to be >95% pure by HPLC analysis. Analyses indicated by the symbols of the elements or functions were within ±0.4% of the theoretical values.

4.1.1. (2R,4R)-methyl 4-hydroxypyrrolidine-2-carboxylate (**4**)

cs-4-Hydroxy-D-proline **3** (1 g, 7.63 mmol) was added to a solution of $SOCl_2$ (0.61 mL, 8.39 mmol) in MeOH (38 mL). The mixture was stirred overnight. Successively, the mixture was concentrated under reduced pressure and filtered over Amberlyst A-21 resin, thus giving compound **4** as a colorless oil (1.1 g, 99%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 4.38–4.30 (br, 1H), 3.79–3.72 (m, 1H), 3.71 (s, 3H), 3.06–2.87 (m, 2H), 2.69–2.59 (br, 2H), 2.32–2.19 (m, 1H), 1.98–1.93 (m, 1H) ppm. Anal. C6H11NO3 (C, H, N).

4.1.2. (2R,4R)-methyl 1-(3,4-dichlorophenylsulfonyl)-4-hydroxypyrrolidine-2-carboxylate (**5**)

To a solution of **4** (1.1 g, 7.63 mmol), Et_3N (2.1 mL, 15.3 mmol) and DMAP (186 mg, 1.53 mmol) in anhydrous CH_2Cl_2 (76 mL), 2,4-dichlorobenzenesulfonyl chloride (1.3 mL, 8.39 mmol) was added at 0 °C. The mixture was allowed to warm to room temperature, and was left overnight stirring under a nitrogen atmosphere. Successively, the mixture was washed with aqueous 5% $NaHCO_3$, 1 N HCl, and brine. The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Compound **5** was isolated by flash chromatography (pet. ether/EtOAc 1:1) thus giving a white solid (1.9 g, 70%). M.p. 104.2–104.9 °C. $[\alpha]^{23}_D$ = +60.7 (*c* = 1.0, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88 (d, *J* = 1.9 Hz, 1H), 7.63 (dd, *J* = 1.9, 8.8 Hz, 1H), 7.55 (d, *J* = 8.8 Hz, 1H), 4.36-4.33 (m, 2H), 3.68 (s, 3H), 3.41–3.31 (m, 3H), 2.21–2.06 (m, 2H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ 173.5 (s), 137.7 (s, 2C), 133.6 (s), 131.1 (d), 129.2 (d), 126.5 (d), 70.7 (d), 59.4 (d), 56.7 (t), 53.0 (q), 38.8 (t) ppm. ESI-MS *m/z* (ES^+) 353.91 [$(M + H)^+$, 100], 375.87 [$(M + Na)^+$, 3.9]. Anal. C12H13Cl2NO5S (C, H, N).

4.1.3. (2R,4S)-methyl 1-((3,4-dichlorophenyl)sulfonyl)-4-hydroxypyrrolidine-2-carboxylate (**6**)

To a solution of **5** (800 mg, 2.26 mmol), PPh_3 (653 mg, 2.49 mmol) and $ClCH_2CO_2H$ (235 mg, 2.49 mmol) in anhydrous toluene (12 mL), DIAD (0.5 mL, 2.49 mmol) was added dropwise at 0 °C. The mixture was allowed to reach room temperature, and then it was irradiated under microwave conditions for 20 min at 90 °C. Successively, the mixture was concentrated under reduced pressure. The crude product was dissolved in MeOH (7.5 mL) and $NaHCO_3$ (40 mg, 0.45 mmol) was added. The mixture was irradiated under microwave conditions for 15 min at 90 °C. The mixture was concentrated under reduced pressure, then the crude dissolved in EtOAc and washed with aqueous 5% $NaHCO_3$ and brine. The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Compound **6** was isolated by flash chromatography (pet. ether/EtOAc 3:1), thus giving a colorless oil (720 mg, 90% over two steps). $[\alpha]^{23}_D$ = +92.3 (*c* = 0.95, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 (d, *J* = 1.9 Hz, 1H), 7.70 (dd, *J* = 1.9, 8.8 Hz, 1H), 7.58 (d, *J* = 8.8 Hz, 1H), 4.45–4.39 (m, 2H), 3.73 (s, 3H), 3.55 (dd, *J* = 3.9, 11.7 Hz, 1H), 3.43 (d, *J* = 11.7 Hz, 1H), 2.29–2.23 (m, 2H), 2.11-2.05 (m, 1H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ 171.9 (s), 137.5 (s), 137.3 (s), 133.2 (s), 130.6 (d), 129.2 (d), 126.4 (d), 69.6 (d), 59.3 (d), 56.3 (t), 52.4 (q), 39.2 (t) ppm. ESI-MS *m/z* (ES^+) 353.82 [$(M + H)^+$, 45.7], and 375.93 [$(M + Na)^+$, 24.02]. Anal. C12H13Cl2NO5S (C, H, N).

4.1.4. (2R,4R)-methyl 4-(benzyloxy)-1-((3,4-dichlorophenyl) sulfonyl)pyrrolidine-2-carboxylate (**7**)

To a solution of **5** (100 mg, 0.28 mmol), benzyl 2,2,2-trichloroacetimidate (105 μL, 0.56 mmol) and 3Å MS (200 mg) in anhydrous Et_2O (2.8 mL) was added 30 μL of trifluoromethansulfonic acid (0.34 mmol) at 0 °C. The mixture was allowed to reach room temperature, then it was left stirring under a nitrogen atmosphere for 3 h. Successively, the mixture was washed with aqueous 5% $NaHCO_3$ and brine. The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Compound **7** was isolated by flash chromatography (pet. ether/EtOAc 2:1) to give a white solid (125 mg, 99%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.95 (d, *J* = 1.9 Hz, 1H), 7.68 (dd, *J* = 1.9, 8.8 Hz, 1H), 7.51 (d, *J* = 8.8 Hz, 1H), 7.25–7.13 (m, 5H), 4.55 (dd, *J* = 2.4, 9.2 Hz, 1H), 4.34 (q, *J* = 11.7 Hz, 2H), 4.09–4.05 (m, 1H), 3.55 (s, 3H), 3.53-3.50 (m, 1H), 3.30 (dd, *J* = 2.4, 10.7 Hz, 1H), 2.43 (d, *J* = 10.7 Hz, 1H), 2.15–2.08 (m, 1H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ 171.4 (s),

138.5 (s), 136.8 (s), 136.4 (s), 133.5 (s), 130.6 (d), 129.2 (d), 128.1 (d, 2C), 127.6 (d), 127.1 (d, 2C), 126.3 (d), 76.7 (t), 70.6 (d), 59.3 (d), 52.9 (t), 52.2 (q), 35.6 (t) ppm. ESI-MS *m/z* (ES^+) 443.15 [M^+, 21.3], 466.09 [$(M + Na)^+$, 100]. Anal. C19H19Cl2NO5S (C, H, N).

4.1.5. (2R,4S)-methyl 4-(benzyloxy)-1-(3,4-dichlorophenylsulfonyl) pyrrolidine-2-carboxylate (**8**)

Prepared as reported for **7** starting from **6** (100 mg, 0.28 mmol). Compound **8** was isolated by flash chromatography (pet. ether/EtOAc 2:1) thus giving a colourless oil (125 mg, 99%). $[\alpha]^{23}_D = +84.65$ ($c = 0.85$, CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$): δ 7.95 (d, $J = 1.9$ Hz, 1H), 7.66 (dd, $J = 1.9$, 8.8 Hz, 1H), 7.45 (d, $J = 8.8$ Hz, 1H), 7.33–7.25 (m, 3H), 7.06 (d, $J = 7.8$ Hz, 2H), 4.32 (t, $J = 7.8$ Hz, 1H), 4.28 (s, 2H), 4.12–4.11 (br, 1H), 3.77 (s, 3H), 3.59 (d, $J = 2.4$ Hz, 2H), 2.41–2.36 (m, 1H), 2.12-2.06 (m, 1H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ 172.0 (s), 137.5 (s, 2C), 136.8 (s), 133.4 (s), 130.8 (d), 129.4 (d), 128.4 (d, 2C), 127.8 (d), 127.1 (d, 2C), 126.6 (d), 76.6 (t), 70.8 (d), 59.9 (d), 55.7 (t), 52.7 (q), 37.0 (t) ppm. ESI-MS *m/z* (ES^+) 443.15 [M^+, 18.1], 466.09 [$(M + Na)^+$, 100]. Anal. C19H19Cl2NO5S (C, H, N).

4.1.6. (2R,4S)-methyl 4-azido-1-(3,4-dichlorophenylsulfonyl) pyrrolidine-2-carboxylate (**9**)

To a solution of **5** (250 mg, 0.70 mmol), Et_3N (118 μL, 0.84 mmol) in anhydrous CH_2Cl_2 (4.2 mL), methanesulfonyl chloride (55 μL, 0.84 mmol) was added at 0 °C. The mixture was allowed to reach room temperature, and was left stirring under a nitrogen atmosphere for 2 h. Successively, the mixture was washed with $NaHCO_3$ and brine, then the organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude was dissolved in DMF (1.7 mL) and NaN_3 (92 mg, 1.40 mmol) was added. The mixture was heated at 90 °C overnight. Successively, the mixture was diluted with EtOAc, and washed with H_2O and brine. The organic phase was dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. Compound **9** was isolated by flash chromatography (pet. ether/EtOAc 1:1) to give a white solid (200 mg, 75% over two steps). M.p. 119.7–120.6 °C. $[\alpha]^{23}_D = +53.6$ ($c = 0.95$, CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$): δ 7.95 (d, $J = 1.9$ Hz, 1H), 7.69 (dd, $J = 1.9$, 8.8 Hz, 1H), 7.60 (d, $J = 8.8$ Hz, 1H), 4.34 (t, $J = 7.8$ Hz, 1H), 4.23–4.19 (m, 1H), 3.74 (s, 3H), 3.64 (dd, $J = 4.8$, 11.2 Hz, 1H), 3.46 (ddd, $J = 1.4$, 2.9, 11.2 Hz, 1H), 2.29–2.16 (m, 2H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ 171.1 (s), 137.3 (s), 137.1 (s), 133.3 (s), 130.7 (d), 129.0 (d), 126.2 (d), 59.1 (d), 59.0 (d), 52.9 (t), 52.5 (q), 36.1 (t) ppm. ESI-MS *m/z* (ES^+) 401.04 [$(M + Na)^+$, 100]. Anal. C12H12Cl2N4O4S (C, H, N).

4.1.7. (2R,4R)-methyl 4-azido-1-(3,4-dichlorophenylsulfonyl) pyrrolidine-2-carboxylate (**10**)

Prepared as reported for **9** starting from **6** (250 mg, 0.70 mmol). Compound **10** was isolated by flash chromatography (pet. ether/EtOAc 1:1), thus giving a white solid (203 mg, 77% over two steps). M.p. 69.8–70.9 °C. $[\alpha]^{23}_D = +29.5$ ($c = 1.0$, CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$): δ 8.02 (d, $J = 1.9$ Hz, 1H), 7.77 (dd, $J = 1.9$, 8.8 Hz, 1H), 7.60 (d, $J = 8.8$ Hz, 1H), 4.62–4.59 (m, 1H), 4.21–4.18 (m, 1H), 3.72 (s, 3H), 3.68 (dd, $J = 5.8$, 10.7 Hz, 1H), 3.26 (dd, $J = 3.4$, 10.7 Hz, 1H), 2.46-2.93 (m, 1H), 2.31-2.26 (m, 1H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ 170.6 (s), 138.2 (s), 137.5 (s), 133.2 (s), 130.7 (d), 129.2 (d), 126.3 (d), 59.0 (d), 58.9 (d), 52.4 (t), 52.2 (q), 35.7 (t) ppm. ESI-MS *m/z* (ES^+) 401.04 [$(M + Na)^+$, 100]. Anal. C12H12Cl2N4O4S (C, H, N).

4.1.8. (2R,4S)-methyl 1-(3,4-dichlorophenylsulfonyl)-4-(4-phenyl-1H-1,2,3-triazol-1-yl)pyrrolidine-2-carboxylate (**11**)

To a solution of **9** (100 mg, 0.26 mmol) and ethynylbenzene (27 mg, 0.26 mmol) in H_2O/*t*BuOH (0.7 mL/0.7 mL) a solution of 0.9 M sodium ascorbate (0.29 mL, 0.26 mmol) and a solution of 0.3 M $Cu(OAc)_2$ (0.87 mL, 0.26 mmol) were added. The mixture was stirred overnight at room temperature. Successively, the mixture was diluted with EtOAc, and washed with aqueous $NaHCO_3$ and brine. The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Compound **11** was isolated by flash chromatography (pet. ether/EtOAc 1:1) thus giving a white solid (91 mg, 73%). M.p. 160.5–161.4 °C. $[\alpha]^{23}_D = +42.6$ ($c = 0.75$, CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$): δ 7.95 (d, $J = 1.9$ Hz, 1H), 7.76–7.73 (m, 3H), 7.59 (dd, $J = 1.9$, 8.8 Hz, 1H), 7.52 (d, $J = 8.8$ Hz, 1H), 7.43–7.39 (m, 2H), 7.36–7.32 (m, 1H), 5.25–5.22 (m, 1H), 4.69 (t, $J = 7.8$ Hz, 1H), 4.06 (dd, $J = 5.8$, 10.7 Hz, 1H), 3.88 (dd, $J = 3.9$, 10.7 Hz, 1H), 3.79 (s, 3H), 3.04–2.97 (m, 1H), 2.65–2.58 (m, 1H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ 171.5 (s), 147.9 (s), 137.9 (s), 137.1 (s), 133.4 (s), 130.9 (d), 129.4 (d), 128.7 (d), 128.5 (d, 2C), 128.1 (d), 125.9 (d), 125.3 (d, 2C), 117.8 (d), 59.3 (d), 58.1 (d), 53.5 (t), 52.6 (q), 36.1 (t) ppm. ESI-MS *m/z* (ES^+) 480.99 [$(M+1)^+$, 31.37], 502.98 [$(M + Na)^+$, 100]. Anal. C20H18Cl2N4O4S (C, H, N).

4.1.9. (2R,4R)-methyl 1-(3,4-dichlorophenylsulfonyl)-4-(4-phenyl-1H-1,2,3-triazol-1-yl)pyrrolidine-2-carboxylate (**12**)

Prepared as reported for **11** starting from **10** (100 mg, 0.26 mmol). Compound **12** was isolated by flash chromatography (pet. ether/EtOAc 1:1) thus giving a white solid (70 mg, 56%). M.p. 177.3–178.0 °C. $[\alpha]^{23}_D = +2.0$ ($c = 0.45$, CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$): δ 7.99 (d, $J = 1.9$ Hz, 1H), 7.87 (s, 1H), 7.73–7.70 (m, 3H), 7.57 (d, $J = 8.2$ Hz, 1H), 7.37–7.33 (m, 2H), 7.29–7.25 (m, 1H), 5.22–5.15 (m, 1H), 4.56 (dd, $J = 5.3$, 9.3 Hz, 1H), 3.97 (dd, $J = 7.0$, 10.8 Hz, 1H), 3.71 (dd, $J = 5.3$, 10.8 Hz, 1H), 3.65 (s, 3H), 2.96–2.88 (m, 1H), 2.64–2.58 (m, 1H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ 170.7 (s), 138.3 (s), 137.6 (s, 2C), 133.8 (s), 131.2 (d), 130.0 (s), 129.5 (d), 128.8 (d, 2C), 128.3 (d, 2C), 126.6 (d, 2C), 125.6 (d, 2C), 118.3 (d, 2C), 59.1 (d), 58.1 (d), 53.0 (t + q, 2C), 36.6 (t) ppm. ESI-MS *m/z* (ES^+) 481.09 [$(M+1)^+$, 27.73], 502.94 [$(M + Na)^+$, 100]. Anal. C20H18Cl2N4O4S (C, H, N).

4.1.10. (2R,4S)-methyl 4-amino-1-(3,4-dichlorophenylsulfonyl) pyrrolidine-2-carboxylate (**13**)

Compound **9** (400 mg, 1.06 mmol) was dissolved in MeOH (5 mL), and 10% Pd/C (53 mg) was added. The resulting mixture was stirred overnight at room temperature under a hydrogen atmosphere. The suspension was then filtered over Celite, and MeOH was removed under reduced pressure. The mixture was filtered over Amberlyst A-21 resin, thus giving compound **13** as a colorless oil, which was used directly for subsequent steps (363 mg, 97%). ^1H NMR (200 MHz, $CDCl_3$): δ 7.88 (d, $J = 6.9$ Hz, 1H), 7.63–7.48 (m, 2H), 4.40 (dd, $J = 5.4$, 8.4 Hz, 1H), 3.70 (s, 3H), 3.66–3.60 (m, 2H), 3.03–2.99 (m, 1H), 2.20–2.08 (m, 1H), 1.94–1.80 (m, 1H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ 171.6 (s), 137.2 (s), 137.2 (s), 133.4 (s), 130.6 (d), 129.2 (d), 126.3 (d), 58.3 (d), 51.9 (q), 51.2 (t), 45.4 (d), 33.5 (t) ppm. Anal. C12H14Cl2N2O4S (C, H, N).

4.1.11. (2R,4R)-methyl 4-amino-1-(3,4-dichlorophenylsulfonyl) pyrrolidine-2-carboxylate (**14**)

Prepared as reported for **13** starting from **10** (400 mg, 1.06 mmol). After filtration of the mixture over Amberlyst A-21 resin, compound **14** was obtained as a colorless oil, and directly used for subsequent steps (358 mg, 96%). ^1H NMR (200 MHz, $CDCl_3$): δ 7.91 (d, $J = 6.9$ Hz, 1H), 7.61–7.49 (m, 2H), 4.54 (dd, $J = 4.7$, 8.7 Hz, 1H), 4.21 (t, $J = 5.8$ Hz, 1H) 3.89 (dd, $J = 5.8$, 10.9 Hz, 1H), 3.71 (s, 3H), 3.48 (dd, $J = 4.7$, 10.9 Hz, 1H), 2.69–2.54 (m, 1H), 2.44–2.32 (m, 1H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ 171.9 (s), 137.6 (s), 137.0 (s), 133.2 (s), 130.5 (d), 129.1 (d), 126.4 (d), 57.7 (d), 52.3 (q), 50.9 (t), 45.1 (d), 33.2 (t) ppm. Anal. C12H14Cl2N2O4S (C, H, N).

4.1.12. (2R,4S)-methyl 1-(3,4-dichlorophenylsulfonyl)-4-(2,4-dimethoxybenzamido)pyrrolidine-2-carboxylate (**15**)

To a solution of **13** (50 mg, 0.14 mmol) and Et_3N (39 μL, 0.28 mmol) in anhydrous CH_2Cl_2 (0.7 mL) 2,4-dimethoxybenzoyl

chloride (28 mg, 0.14 mmol) was added at 0 °C. The mixture was allowed to warm to room temperature, and it was left overnight stirring under a nitrogen atmosphere. Successively, the mixture was washed with $NaHCO_3$, 1 N HCl and brine. The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Compound **15** was isolated by flash chromatography (pet. ether/EtOAc 1:2) thus giving a white solid (44 mg, 60%). M.p. 75.5–76.5 °C. $[\alpha]^{22}_D = +69.23$ ($c = 0.3$, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.07 (d, $J = 8.7$ Hz, 1H), 7.97 (d, $J = 6.3$ Hz, 1H), 7.89 (d, $J = 6.8$ Hz, 1H), 7.47-7.43 (m, 2H), 6.55 (dd, $J = 1.9$, 8.7 Hz, 1H), 6.44 (d, $J = 1.9$ Hz, 1H), 4.64–4.62 (br, 1H), 4.58 (t, $J = 7.8$ Hz, 1H), 3.91 (s, 3H), 3.82 (s, 3H), 3.65 (s, 3H), 3.59-5.58 (m, 2H), 2.44–2.38 (m, 1H), 2.29–2.22 (m, 1H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ 171.9 (s), 164.8 (s), 163.5 (s), 158.8 (s, 2C), 138.7 (s), 133.6 (d), 133.7 (d), 128.8 (s + d, 2C), 127.4 (d), 113.5 (s), 105.2 (d), 98.4 (d), 58.9 (d), 56.0 (q), 55.6 (q), 53.5 (d), 52.6 (t), 49.7 (q), 37.6 (t) ppm. Anal. C21H22Cl2N2O7S (C, H, N).

4.1.13. (2R,4S)-methyl 1-(3,4-dichlorophenylsulfonyl)-4-(thiophene-2-carboxamido)pyrrolidine-2-carboxylate (**18**)

To a solution of **13** (100 mg, 0.28 mmol) and Et_3N (79 μL, 0.57 mmol) in anhydrous CH_2Cl_2 (1.4 mL) thiophene-2-carbonyl chloride (41 mg, 0.28 mmol) was added at 0 °C. The mixture was allowed to reach room temperature, and it was left overnight stirring under a nitrogen atmosphere. Successively, the mixture was washed with aqueous $NaHCO_3$, 1 N HCl and brine. The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Compound **18** was isolated by flash chromatography (pet. ether/EtOAc 1:2) thus giving a yellow oil (32 mg, 25%). $[\alpha]^{22}_D = +59.5$ ($c = 0.25$, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) major rotamer: δ 7.85 (d, $J = 6.8$ Hz, 2H), 7.46–7.39 (m, 4H), 7.37–7.34 (m, 1H), 6.98–6.93 (m, 1H), 6.58 (d, $J = 6.3$ Hz, 1H), 4.56 (t, $J = 7.8$ Hz, 2H), 3.61 (s, 3H), 3.54–3.50 (m, 2H), 2.38–2.31 (m, 1H), 2.25–2.18 (m, 1H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) major rotamer: δ 171.6 (s), 161.6 (s), 138.1 (s), 138.0 (s), 133.1 (d), 130.5 (d), 129.0 (d), 128.7 (s), 128.3 (d), 127.6 (d, 2C), 126.6 (s), 58.8 (d), 53.3 (t), 52.7 (q), 50.0 (d), 37.1 (t) ppm. Anal. C17H16Cl2N2O5S2 (C, H, N).

4.1.14. (2R,4R)-methyl 1-(3,4-dichlorophenylsulfonyl)-4-(2,4-dimethoxybenzamido)pyrrolidine-2-carboxylate (**16**)

Prepared as reported for **15** starting from **14** (75 mg, 0.21 mmol). Compound **16** was isolated by flash chromatography (pet. ether/EtOAc 1:2) thus giving a white solid (44 mg, 40%). M.p. 68.3–69.2 °C. $[\alpha]^{22}_D = +16.2$ ($c = 0.2$, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.46 (d, $J = 8.3$ Hz, 1H), 8.07 (d, $J = 8.7$ Hz, 1H), 7.87 (d, $J = 7.3$ Hz, 1H), 7.58 (d, $J = 7.3$ Hz, 1H), 7.52 (t, $J = 7.3$ Hz, 1H), 6.53 (dd, $J = 2.4$, 8.7 Hz, 1H), 6.44 (d, $J = 2.4$ Hz, 1H), 4.79–4.75 (br, 1H), 4.29 (dd, $J = 3.4$, 10.2 Hz, 1H), 3.93 (s, 3H), 3.80 (s, 3H), 3.71 (s, 3H), 3.51 (dd, $J = 5.8$, 10.2 Hz, 1H), 3.44 (dd, $J = 2.4$, 10.2 Hz, 1H), 2.39–2.31 (m, 1H), 2.06–2.01 (m, 1H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ 172.6 (s), 164.5 (s), 163.4 (s), 159.0 (s, 2C), 137.0 (s), 133.7 (d), 133.0 (d), 129.1 (d), 128.8 (s), 127.5 (d), 113.6 (s), 105.1 (d), 98.3 (d), 59.2 (d), 55.8 (q), 55.6 (q), 55.0 (d), 52.6 (t), 48.4 (q), 36.9 (t) ppm. Anal. C21H22Cl2N2O7S (C, H, N).

4.1.15. (2R,4S)-methyl 1-(3,4-dichlorophenylsulfonyl)-4-((E)-3-(furan-2-yl)acrylamido)pyrrolidine-2-carboxylate (**17**)

To a solution of **14** (100 mg, 0.28 mmol) and Et_3N (79 μL, 0.57 mmol) in anhydrous CH_2Cl_2 (1.4 mL) (*E*)-3-(furan-2-yl)acryloyl chloride (44 mg, 0.28 mmol) was added at 0 °C. The mixture was allowed to reach room temperature, and it was left overnight stirring under a nitrogen atmosphere. Successively, the mixture was washed with aqueous $NaHCO_3$, 1 N HCl and brine. The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Compound **17** was isolated by flash chromatography (pet. ether/EtOAc 1:2) thus giving a brown solid (49 mg, 37%). M.p. 69.4–70.2 °C. $[\alpha]^{22}_D = +45.2$ ($c = 0.3$, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82 (d, $J = 6.8$ Hz, 1H), 7.48–7.41 (m, 2H), 7.35–7.34 (br, 1H), 7.20 (d, $J = 15.1$ Hz, 1H), 6.45 (d, $J = 3.4$ Hz, 1H), 6.35 (dd, $J = 1.9$, 3.4 Hz, 1H), 6.27 (d, $J = 6.3$ Hz, 1H), 6.00 (d, $J = 15.1$ Hz, 1H), 4.49 (t, $J = 7.8$ Hz, 2H), 3.60 (s, 3H), 3.58–3.57 (m, 1H), 3.43 (dd, $J = 2.9$, 10.7 Hz, 1H), 2.25-2.19 (m, 2H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ 171.7 (s), 165.6 (s), 150.9 (s, 2C), 144.0 (d), 137.9 (s), 133.1 (d), 129.1 (d), 128.0 (d), 127.4 (d + s, 2C), 117.7 (d), 114.0 (d), 112.1 (d), 59.3 (d), 53.5 (t), 52.6 (q), 49.5 (d), 36.8 (t) ppm. Anal. C19H18Cl2N2O6S (C, H, N).

4.1.16. (2R,4R)-methyl 1-(3,4-dichlorophenylsulfonyl)-4-(thiophene-2-carboxamido)pyrrolidine-2-carboxylate (**19**)

Prepared as reported for **18** starting from **14** (100 mg, 0.28 mmol). Compound **19** was isolated by flash chromatography (pet. ether/EtOAc 1:2) thus giving a white solid (54 mg, 42%). M.p. 56.8–58.2 °C. $[\alpha]^{22}_D = +51.9$ ($c = 0.3$, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) major rotamer: δ 7.79 (d, $J = 6.8$ Hz, 1H), 7.68 (d, $J = 9.2$ Hz, 1H), 7.54 (d, $J = 7.8$ Hz, 1H), 7.47 (t, $J = 8.2$ Hz, 2H), 7.41 (dd, $J = 0.9$, 4.8 Hz, 1H), 7.01 (t, $J = 4.8$ Hz, 1H), 4.71–4.69 (m, 1H), 4.20 (dd, $J = 1.9$, 10.2 Hz, 1H), 3.73 (s, 3H), 3.51 (d, $J = 9.7$ Hz, 1H), 3.30 (dd, $J = 4.8$, 9.7 Hz, 1H), 2.27–2.20 (m, 1H), 1.95 (dd, $J = 1.9$, 12.1 Hz, 1H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) major rotamer: δ 174.0 (s), 160.9 (s), 138.6 (s), 136.4 (s), 133.3 (d), 130.4 (d), 129.2 (d), 129.0 (s), 128.2 (s), 128.1 (d), 127.7 (d), 127.5 (d), 58.9 (d), 55.3 (t), 53.3 (q), 48.9 (d), 36.6 (t) ppm. Anal. C17H16Cl2N2O5S2 (C, H, N).

4.1.17. Ethyl 3-((tert-butoxycarbonyl)(2-ethoxy-2-oxoethyl)amino)propanoate (**30**)

To a suspension of ethyl glycinate hydrochloride **29** (7.0 g, 50.1 mmol) in 95% ethanol (115 mL) was added ethyl acrylate (6.0 mL, 55.1 mmol) and Et_3N (7.0 mL, 50.1 mmol). The reaction mixture was allowed to stir at room temperature for 48 h. The reaction was concentrated and water was added. The aqueous phase was extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4 and evaporated. The crude product, isolated as a yellow oil, was dissolved in $CHCl_3$ (80 mL), and Boc_2O (15.3 g, 70.1 mmol) was added at 0 °C, followed by 5% aqueous NaOH (80 mL). The reaction was stirred at 0 °C for 1 h, and then at room temperature for 72 h. The mixture was extracted with $CHCl_3$ and the organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by automated flash chromatography (pet. ether/EtOAc 4:1, R_f 0.56), to afford **30** as a yellow oil (11.78 g, 77% yield over two step). ^{1}H NMR ($CDCl_3$, 200 MHz) major rotamer: δ 4.16 (q, $J = 7.1$ Hz, 2H), 4.11 (qd, $J = 3.6$, 7.1 Hz, 2H), 4.00 and 3.94 (s, 2H), 3.55 and 3.51 (t, $J = 6.6$ Hz, 2H), 2.62 and 2.59 (t, $J = 6.6$ Hz, 2H), 1.46 and 1.40 (s, 9H), 1.25 (m, 6H). Anal. C14H25NO6 (C, H, N).

4.1.18. (R/S)-1-tert-Butyl 2-ethyl 3-oxopyrrolidine-1,2-dicarboxylate (**31**)

To a suspension of potassium *tert*-butoxide (791 mg, 7.05 mmol) in anhydrous toluene (19 mL) at −10 °C a solution of **30** (1.43 g, 4.7 mmol) in toluene was added dropwise. The reaction was stirred at 0 °C for 90 min and then it was quenched with acetic acid (500 μL), followed by the addition of a cold solution of $NaH_2PO_4 \cdot H_2O$ (2.5 g) in 25 mL of water. The layers were separated, and the aqueous phase was extracted with $CHCl_3$. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash chromatography (silica gel, pet. ether/EtOAc 5:1, R_f 0.44) obtaining the title compound **31** as a yellow oil (497 mg, 41% yield). ^{1}H NMR ($CDCl_3$, 200 MHz) major rotamer: δ 4.55 and 4.47 (s, 1H), 4.27–4.22 (m, 2H), 3.91-3.84 (m) and 3.82 (t, $J = 8.2$ Hz) 2H, 2.69 (t, $J = 8.0$ Hz,

2H), 1.49 and 1.43 (s, 9H), 1.30 (t, $J = 7.1$ Hz, 3H). Anal. C12H19NO5 (C, H, N).

4.1.19. (2R,3S)-1-tert-Butyl 2-ethyl 3-hydroxypyrrolidine-1,2-dicarboxylate (**32**)

Immobilized baker yeast preparation: separate solutions of dried baker's yeast (4.0 g) and sodium alginate (971 mg), each in 40 mL of water were prepared by slow addition of reagent into water. When both reagents were completely dissolved, the two solutions were combined and added dropwise by means of an addition funnel to a solution of 10% calcium chloride (137 mL). Upon impact with calcium chloride solution, the drops formed gelatinous beads, containing the baker's yeast. The beads were washed three times with 130 mL of water and used for the reaction. The beads were placed in a 500 mL round flask and 31 mL of water were added. Sucrose (5.8 g) was added, the mixture was stirred at room temperature for 30 min, and then **31** (385 mg, 1.5 mmol) was added. The reaction was stirred at 30 °C for 48 h. The reaction mixture was then filtered, and yeast beads were washed twice with Et_2O (40 mL). The aqueous layer was centrifuged, and then extracted with Et_2O. The combined organic extracts were dried over anhydrous Na_2SO_4 and evaporated. The crude product was purified by automated flash chromatography (pet. ether/EtOAc 1:1, R_f 0.35), affording desired compound **32** as a yellow oil (185 mg, 47% yield). $[\alpha]^{24}_D = +29.0$ ($c = 1.0$, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz) major rotamer: δ 4.6–4.58 (m, 1H), 4.31 (d, $J = 6.9$ Hz, 1H), 4.24–4.17 (m, 2H), 3.68–3.56 (m, 1H), 3.52–3.43 (m, 1H), 2.62 (br, 1H), 2.14–1.96 (m, 2H), 1.40 (s, 9H), 1.31–1.25 (m, 3H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) major rotamer: δ 170.5 (s), 154.0 (s), 80.0 (s), 72.0 (d), 63.9 (d), 60.8 (t), 43.7 (t), 31.9 (t), 28.1 (q), 14.2 (q) ppm. ESI-MS m/z (ES^+) 259.65 [$(M+1)^+$, 18.34], 281.12 [$(M + Na)^+$, 24.52], 540.84 [$(2M + Na)^+$, 100]. Anal. C12H21NO5 (C, H, N).

4.1.20. (2R,3S)-1-tert-Butyl 2-ethyl 3-(benzyloxy)pyrrolidine-1,2-dicarboxylate (**33**)

To a stirred solution of **32** (317 mg, 1.22 mmol) in dry THF, benzyl bromide (173 μL, 1.46 mmol) and TBAI (137 mg, 0.37 mmol) were added under N_2 atmosphere. The mixture was cooled to 0 °C, and 60% NaH (58 mg, 1.46 mmol) was added. The reaction mixture was stirred at room temperature overnight. The mixture was poured into ice water and extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by automated flash chromatography (pet. ether/EtOAc 2:1, R_f 0.28) affording **33** as colorless oil (41% yield). $[\alpha]^{24}_D$ −41.3 ($c = 0.45$, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz) major rotamer: δ 7.37–7.26 (m, 5H), 4.71–4.46 (m, 3H), 4.18 (q, $J = 7.1$ Hz, 2H), 3.77–3.59 (m, 2H), 3.40–3.32 (m, 1H), 2.20–2.03 (m, 2H), 1.42 (s, 9H), 1.24 (t, $J = 7.1$ Hz, 3H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) major rotamer: δ 169.7 (s), 153.4 (s), 137.2 (s), 127.9 (d), 127.3 (d), 127.0 (d), 79.7 (d), 78.4 (s), 71.7 (t), 61.7 (d), 60.5 (t), 43.1 (t), 28.8 (t), 28.0 (q), 14.0 (q) ppm. ESI-MS m/z (ES^+) 349.93 [$(M+1)^+$, 28.24], 372.09 [$(M + Na)^+$, 100]. Anal. C19H27NO5 (C, H, N).

4.1.21. (2R,3S)-1-tert-Butyl 2-ethyl 3-(hexyloxy)pyrrolidine-1,2-dicarboxylate (**34**)

Compound **32** (148 mg, 0.6 mmol) was dissolved in DMF (1.8 mL) and 1-iodohexane (177 μL, 1.2 mmol) was added. The mixture was cooled to 0 °C, and 60% NaH (72 mg, 1.8 mmol) was added. The reaction mixture was allowed to reach room temperature, and it was stirred overnight. The mixture was then diluted with H_2O and extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and evaporated. A yellow oil was obtained and purified by automated flash chromatography (EP/Et_2O 3:1, R_f 0.28), giving the title compound **34** as a colorless oil (130 mg, 66% yield). $[\alpha]^{24}_D$ −13.0 ($c = 1.14$, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) major rotamer: δ 4.40 (d, $J = 7.1$ Hz, 1H), 4.21–4.15 (m, 2H), 3.69–3.58 (m, 1H), 3.56–3.29 (m, 2H + 2H), 2.07–2.0 (m, 2H), 1.53–1.47 (m, 2H), 1.40 (s, 9H), 1.30–1.23 (m, 9H), 0.89–0.85 (m, 3H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) major rotamer: δ 169.9 (s), 153.7 (s), 79.7 (s), 79.6 (d), 78.8 (s), 70.6 (t), 62.0 (d), 60.5 (t), 43.3 (t), 31.5 (t), 29.5 (t), 29.0 (t), 28.2 (q), 25.6 (t), 22.4 (t), 14.2 (q), 13.8 (q) ppm. Anal. C18H33NO5 (C, H, N).

4.1.22. (2R,3S)-ethyl 3-(benzyloxy)-1-((3,4-dichlorophenyl) sulfonyl)pyrrolidine-2-carboxylate (**35**)

To a stirred solution of **33** (352 mg, 1.01 mmol) in CH_2Cl_2 (10 mL) was added TFA (10 mL). The reaction mixture was stirred at room temperature for 3 h. After evaporation of the solvent and filtration over Amberlyst A-21 resin, crude product, isolated as a yellow oil, was used in the next step without further purification. To a suspension of crude in dry CH_2Cl_2 (13.6 mL) Et_3N (281 μL, 2.02 mmol) and DMAP (24 mg, 0.2 mmol) were added under N_2 atmosphere. The mixture was cooled to 0 °C and 3,4-dichlorobenzylsulfonyl chloride (272 mg, 1.11 mmol) was added in portions. The mixture was allowed to reach room temperature and stirred overnight. Then it was washed with a saturated $NaHCO_3$ solution, 5% HCl and brine. The organic layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The crude product was purified by automated flash chromatography (pet. ether/EtOAc 4:1, R_f 0.51) to obtain 306 mg of **35** as a yellow oil (67% yield). $[\alpha]^{24}_D$ −11.8 ($c = 1.0$, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.95 (d, $J = 2.2$ Hz, 1H), 7.71 (dd, $J = 2.2$, 8.5 Hz, 1H), 7.58 (d, $J = 8.5$ Hz, 1H), 7.36–7.24 (m, 5H), 4.65–4.60 (m, 3H), 4.31–4.14 (m, 3H), 3.53–3.45 (m, 2H), 2.18–2.04 (m, 2H), 1.25–1.17 (m, 3H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ 168.4 (s), 138.4 (s), 137.2 (s), 136.8 (s), 133.1 (s), 130.7 (d), 128.9 (d), 128.1 (d), 127.6 (d), 127.2 (d) 126.2 (d), 78.3 (d), 72.0 (t), 62.6 (d), 61.2 (t), 45.3 (t), 29.8 (t), 13.8 (q) ppm. Anal. C20H21Cl2NO5S (C, H, N).

4.1.23. (2R,3S)-ethyl 3-(benzyloxy)-1-((4-chlorophenyl)sulfonyl) pyrrolidine-2-carboxylate (**36**)

Prepared as reported for **35** starting from **33** (352 mg, 1.01 mmol) and 4-chlorobenzylsulfonyl chloride (325 mg, 1.54 mmol). The crude product was purified by automated flash chromatography (pet. ether/EtOAc 4:1, R_f 0.49) to give compound **36** as a yellow oil (157 mg, 26% yield). $[\alpha]^{24}_D$ −18.0 ($c = 1.0$, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.82 (dd, $J = 1.9$ Hz, $J = 6.6$ Hz, 2H), 7.48 (dd, $J = 1.9$, 6.6 Hz, 2H), 7.33–7.24 (m, 5H), 4.63–4.53 (m, 2H), 4.24 (q, $J = 7.1$ Hz, 2H), 4.20–4.03 (m, 2H), 3.58–3.39 (m, 2H), 2.21–2.01 (m, 2H), 1.18 (t, $J = 7.1$ Hz, 3H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ 168.8 (s), 139.2 (s), 137.2 (s), 137.1 (s), 129.2 (d), 128.8 (d), 128.4 (d), 127.8 (d), 127.4(d), 78.5 (d), 72.3 (t), 62.7 (d), 61.3 (t), 45.4 (t), 30.0 (t), 14.0 (q) ppm. Anal. C20H22ClNO5S (C, H, N).

4.1.24. (2R,3S)-ethyl 1-((4-chlorophenyl)sulfonyl)-3-(hexyloxy) pyrrolidine-2-carboxylate (**37**)

Prepared as reported for **35** starting from compound **34** (130 mg, 0.4 mmol) and 4-chlorobenzylsulfonyl chloride (93 mg, 0.44 mmol). The crude product was purified by flash cromatography (silica gel, pet. ether/EtOAc 4:1, R_f 0.52) to obtain **37** as a yellow oil (102 mg, 60% yield). $[\alpha]^{28}_D$ −30.2 ($c = 0.7$, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.81 (dd, $J = 1.9$, 6.6 Hz, 2H), 7.48 (dd, $J = 1.9$, 6.6 Hz, 2H), 4.55 (d, $J = 6.9$ Hz, 1H), 4.20–4.0 (m, 3H), 3.55–3.37 (m, 4H), 2.1–1.99 (m, 2H), 1.50–1.45 (m, 2H), 1.30–1.20 (m, 9H), 0.88–0.84 (m, 3H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ 168.8 (s), 139.1 (s), 137.3 (s), 129.2 (d), 128.8 (d), 79.4 (d), 70.9 (t), 62.8 (d), 61.1 (t), 45.4 (t), 31.5 (t), 30.0 (t), 29.5 (t), 25.5 (t9, 22.4 (t), 14.0 (q), 13.9 (q) ppm. Anal. C19H28ClNO5S (C, H, N).

4.2. General procedure for the synthesis of hydroxamic acids [29]

Preparation of NH_2OK/NH_2OH solution: $NH_2OH \cdot HCl$ (8 eq) was solubilized in MeOH (0.4 mL/mmol) by heating to reflux. Most, but not all of the salt dissolved. The solution was cooled to <40 °C, and a solution of KOH (12 eq) in MeOH (0.2 mL) was added in one portion. The resulting suspension was cooled to room temperature before use and was used without prior removal of precipitated material. A solution of ester (1 eq) in NH_2OK/NH_2OH solution previously prepared was stirred at room temperature 3 days. The reaction mixture was taken up in 1N HCl, extracted with EtOAc, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (EtOAc/MeOH 4:1) to afford hydroxamic acids.

4.2.1. (2R,4R)- 4-(Benzyloxy)-1-(3,4-dichlorophenylsulfonyl)-N-hydroxypyrrolidine-2-carboxamide (**20**)

White solid, 38% yield. M.p. 77.4–79.5 °C. $[\alpha]^{23}_D = +29.6$ ($c = 1$, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.44 (br, 1H), 7.88 (s, 1H), 7.62 (d, $J = 8.4$ Hz, 1H), 7.52 (d, $J = 8.4$ Hz, 1H), 7.22–7.17 (m, 5H), 7.10–7.06 (br, 1H), 4.43 (d, $J = 11.4$ Hz, 1H), 4.26 (d, $J = 11.4$ Hz, 1H), 4.23 (d, $J = 9.3$ Hz, 1H), 3.91 (br, 1H), 3.62 (d, $J = 10.8$ Hz, 1H), 3.07 (dd, $J = 4.0$, 10.8 Hz, 1H), 2.53 (d, $J = 10.8$ Hz, 1H), 1.65–1.58 (m, 1H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ 168.5 (s), 138.6 (s), 136.7 (s), 134.9 (s), 134.2 (s), 131.5 (d), 129.5 (d), 128.4 (s, 2C), 127.9 (d), 127.8 (d, 2C), 126.8 (d), 76.2 (t), 70.9 (d), 60.2 (d), 55.1 (t), 34.7 (t) ppm. ESI-MS m/z (ES^+) 466.92 [$(M + Na)^+$, 100]. Anal. C18H18Cl2N2O5S (C, H, N).

4.2.2. (2R,4S)- 4-(Benzyloxy)-1-(3,4-dichlorophenylsulfonyl)-N-hydroxypyrrolidine-2-carboxamide (**21**)

White solid, 23% yield. M.p. 59.8–61.9 °C. $[\alpha]^{23}_D = +82.5$ ($c = 1$, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.09 (s, 1H), 7.80 (d, $J = 8.3$ Hz, 1H), 7.53 (d, $J = 8.4$ Hz, 1H), 7.46-7.42 (m, 4H), 7.18–7.14 (m, 2H), 4.42 (t, $J = 7.8$ Hz, 1H), 4.33 (q, $J = 11.7$ Hz, 2H), 4.20 (br, 1H), 3.80-3.61 (m, 2H), 2.58-2.53 (m, 1H), 2.39-2.36 (m, 1H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ 169.1 (s), 138.1 (s), 136.7 (s), 135.8 (s), 133.5 (s), 131.0 (d), 129.7 (d), 128.3 (s, 2C), 59.8 (d), 55.0 (t), 36.8 (t) ppm. ESI-MS m/z (ES^+) 444.93 [$(M + H)^+$, 24.83], 466.94 [$(M + Na)^+$, 100]. Anal. C18H18Cl2N2O5S (C, H, N).

4.2.3. (2R,4S)-1-(3,4-dichlorophenylsulfonyl)-N-hydroxy-4-(4-phenyl-1H-1,2,3-triazol-1-yl)pyrrolidine-2-carboxamide (**22**)

White solid, 49% yield. M.p. 186.8–188.8 °C. ^{1}H NMR (400 MHz, DMSO): δ 9.13 (br, 1H), 8.41 (s, 1H), 7.87 (d, $J = 1.9$ Hz, 1H), 7.69 (d, $J = 7.0$ Hz, 2H), 7.58 (dd, $J = 1.9$, 8.4 Hz, 1H), 7.51 (d, $J = 8.4$ Hz, 1H), 7.41 (t, $J = 7.6$ Hz, 2H), 7.30 (t, $J = 7.6$ Hz, 1H), 5.22 (br, 1H), 4.32 (t, $J = 7.6$ Hz, 1H), 4.00 (dd, $J = 4.00$, 11.4 Hz, 1H), 3.87 (d, $J = 11.4$ Hz, 1H), 2.93-2.90 (m, 1H), 2.52-2.50 (m, 1H) ppm. ^{13}C NMR (50 MHz, DMSO): δ 167.3 (s), 146.7 (s), 137.4 (s), 136.7 (s), 132.5 (s), 131.5 (d), 130.6 (s), 129.0 (d, 2C), 128.9 (d), 128.1 (d), 127.2 (d), 125.3 (d, 2C), 120.6 (d), 58.9 (d), 58.8 (d), 55.6 (t), 35.5 (t) ppm. ESI-MS m/z (ES^+) 482.87 [$(M + H)^+$, 100]. Anal. C19H17Cl2N5O4S (C, H, N).

4.2.4. (2R,4R)-1-(3,4-dichlorophenylsulfonyl)-N-hydroxy-4-(4-phenyl-1H-1,2,3-triazol-1-yl)pyrrolidine-2-carboxamide (**23**)

White solid, 53% yield. M.p. 75.9–77.8 °C. $[\alpha]^{22}_D = -15.4$ ($c = 0.5$, EtOAc). ^{1}H NMR (400 MHz, DMSO): δ 8.78 (s, 1H), 7.81 (s, 1H), 7.55 (d, $J = 7.8$ Hz, 2H), 7.66 (d, $J = 8.7$ Hz, 1H), 7.38 (t, $J = 7.3$ Hz, 2H), 7.28 (d, $J = 6.8$ Hz, 1H), 7.13 (t, $J = 8.7$ Hz, 1H), 5.00 (t, $J = 6.3$ Hz, 1H), 4.14 (t, $J = 6.3$ Hz, 1H), 3.87 (t, $J = 10.7$ Hz, 1H), 3.60 (dd, $J = 6.3$, 10.7 Hz, 1H), 2.82–2.75 (m, 1H), 2.37–2.31 (m, 1H) ppm. ^{13}C NMR (50 MHz, DMSO): δ 173.8 (s), 159.9 (s), 146.7 (s), 130.9 (s), 129.4 (d), 129.1 (d, 2C), 128.3 (d), 128.1 (d), 126.5 (d), 125.3 (d, 2C), 120.8 (s), 120.7 (s), 117.4 (d), 61.3 (d), 57.9 (d), 53.3 (t), 36.6 (t) ppm. ESI-MSMS m/z (ES^+) 447.36 [$(M + H\text{-}Cl)^+$, 100]. Anal. C19H17Cl2N5O4S (C, H, N).

4.2.5. (2R,4S)- 1-(3,4-dichlorophenylsulfonyl)-4-(2,4-dimethoxybenzamido)-N-hydroxypyrrolidine-2-carboxamide (**24**)

White solid, 49% yield. M.p. 120.4–122.7 °C. $[\alpha]^{22}_D = +12.9$ ($c = 0.65$, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.07 (d, $J = 8.7$ Hz, 1H), 7.97 (d, $J = 6.3$ Hz, 1H), 7.89 (d, $J = 6.8$ Hz, 1H), 7.47–7.43 (m, 2H), 6.55 (dd, $J = 1.9$, 8.7 Hz, 1H), 6.44 (d, $J = 1.9$ Hz, 1H), 4.64–4.62 (br, 1H), 4.58 (t, $J = 7.8$ Hz, 1H), 3.91 (s, 3H), 3.82 (s, 3H), 3.65 (s, 3H), 3.59-5.58 (m, 2H), 2.44–2.38 (m, 1H), 2.29–2.22 (m, 1H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ 178.3 (s), 164.5 (s), 162.9 (s), 158.5 (s), 136.6 (s), 136.5 (s), 133.0 (d), 131.6 (d), 128.3 (d), 127.0 (d), 113.1 (s), 113.2 (s), 104.6 (d), 97.5 (d), 62.4 (d), 55.4 (q), 55.1 (q), 54.1 (d), 49.4 (t), 36.4 (t) ppm. ESI-MS m/z (ES^+) 518.83 [$(M+1)^+$, 51.29], 535.84 [$(M + Na)^+$, 100]. Anal. C20H21Cl2N3O7S (C, H, N).

4.2.6. (2R,4R)- 1-(3,4-dichlorophenylsulfonyl)-4-(2,4-dimethoxybenzamido)-N-hydroxypyrrolidine-2-carboxamide (**25**)

White solid, 23% yield. M.p. 115.9–118.2 °C. $[\alpha]^{22}_D = +9.0$ ($c = 0.45$, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.07 (d, $J = 8.7$ Hz, 1H), 7.97 (d, $J = 6.3$ Hz, 1H), 7.89 (d, $J = 6.8$ Hz, 1H), 7.47-7.43 (m, 2H), 6.55 (dd, $J = 1.9$, 8.7 Hz, 1H), 6.44 (d, $J = 1.9$ Hz, 1H), 4.64–4.62 (br, 1H), 4.58 (t, $J = 7.8$ Hz, 1H), 3.91 (s, 3H), 3.82 (s, 3H), 3.65 (s, 3H), 3.59–5.58 (m, 2H), 2.44–2.38 (m, 1H), 2.29–2.22 (m, 1H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ 168.4 (s), 165.0 (s), 163.6 (s), 159.1 (s), 135.1 (s), 133.7 (s), 133.6 (d, 2C), 129.5 (d), 127.7 (d), 113.5 (s, 2C), 105.3 (d), 98.4 (d), 59.6 (d), 56.2 (q), 55.9 (q), 55.5 (d), 49.0 (t), 34.4 (t) ppm. ESI-MS m/z (ES^+) 518.89 [$(M+1)^+$, 45.34], 535.79 [$(M + Na)^+$, 100]. Anal. C20H21Cl2N3O7S (C, H, N).

4.2.7. (2R,4S)- 1-(3,4-dichlorophenylsulfonyl)-N-hydroxy-4-(thiophene-2-carboxamido)pyrrolidine-2-carboxamide (**26**)

Yellow solid, 60% yield. M.p. 134.8–137.2 °C. $[\alpha]^{22}_D = +29.3$ ($c = 0.6$, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) major rotamer: δ 7.79 (d, $J = 6.8$ Hz, 1H), 7.68 (d, $J = 9.2$ Hz, 1H), 7.54 (d, $J = 7.8$ Hz, 1H), 7.47 (t, $J = 8.2$ Hz, 2H), 7.41 (dd, $J = 0.9$, 4.8 Hz, 1H), 7.01 (t, $J = 4.8$ Hz, 1H), 4.71-4.69 (m, 1H), 4.20 (dd, $J = 1.9$, 10.2 Hz, 1H), 3.73 (s, 3H), 3.51 (d, $J = 9.7$ Hz, 1H), 3.30 (dd, $J = 4.8$, 9.7 Hz, 1H), 2.27–2.20 (m, 1H), 1.95 (dd, $J = 1.9$, 12.1 Hz, 1H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) major rotamer: δ 179.2 (s), 162.0 (s), 138.1 (s), 137.1 (s), 136.0 (d), 132.4 (s), 132.2 (d), 129.8 (s), 128.6 (d, 2C), 127.0 (d, 2C), 62.1 (d), 54.0 (t), 40.0 (dH), 35.2 (t) ppm. ESI-MS m/z (ES^+) 464.09 [$(M+1)^+$, 61.54], 486.13 [$(M + Na)^+$, 100]. Anal. C16H15Cl2N3O5S2 (C, H, N).

4.2.8. (2R,4R)- 1-(3,4-dichlorophenylsulfonyl)-N-hydroxy-4-(thiophene-2-carboxamido)pyrrolidine-2-carboxamide (**27**)

Yellow solid, 30% yield. M.p. 136.3–138.2 °C. $[\alpha]^{22}_D = +17.6$ ($c = 0.35$, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) major rotamer: δ 7.79 (d, $J = 6.8$ Hz, 1H), 7.68 (d, $J = 9.2$ Hz, 1H), 7.54 (d, $J = 7.8$ Hz, 1H), 7.47 (t, $J = 8.2$ Hz, 2H), 7.41 (dd, $J = 0.9$, 4.8 Hz, 1H), 7.01 (t, $J = 4.8$ Hz, 1H), 4.71–4.69 (m, 1H), 4.20 (dd, $J = 1.9$, 10.2 Hz, 1H), 3.73 (s, 3H), 3.51 (d, $J = 9.7$ Hz, 1H), 3.30 (dd, $J = 4.8$, 9.7 Hz, 1H), 2.27–2.20 (m, 1H), 1.95 (dd, $J = 1.9$, 12.1 Hz, 1H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) major rotamer: δ 168.6 (s), 161.4 (s), 138.1 (s), 135.7 (s), 133.1 (d), 130.1 (d), 128.9 (d, 2C), 128.2 (s), 127.3 (s), 127.1 (d), 127.5 (d), 57.9 (d), 55.3 (t), 48.5 (d), 34.7 (t) ppm. ESI-MS m/z (ES^+) 464.06 [$(M+1)^+$, 67.23], 486.11 [$(M + Na)^+$, 100]. Anal. C16H15Cl2N3O5S2 (C, H, N).

4.2.9. (2R,4S)-1-(3,4-dichlorophenylsulfonyl)-4-((E)-3-(furan-2-yl) acrylamido)-N-hydroxypyrrolidine-2-carboxamide (**28**)

Brown solid, 33% yield. M.p. 181.3–183.9 °C. $[\alpha]^{22}_D = -108.8$ ($c = 0.5$, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82 (d, $J = 6.8$ Hz, 1H), 7.48–7.41 (m, 2H), 7.35–7.34 (br, 1H), 7.20 (d, $J = 15.1$ Hz, 1H), 6.45 (d, $J = 3.4$ Hz, 1H), 6.35 (dd, $J = 1.9$, 3.4 Hz, 1H), 6.27 (d,

J = 6.3 Hz, 1H), 6.00 (d, J = 15.1 Hz, 1H), 4.49 (t, J = 7.8 Hz, 2H), 3.60 (s, 3H), 3.58–3.57 (m, 1H), 3.43 (dd, J = 2.9, 10.7 Hz, 1H), 2.25–2.19 (m, 2H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ 168.7 (s), 165.9 (s), 150.9 (s, 2C), 143.6 (d), 136.3 (s), 132.7 (d), 129.0 (d), 128.2 (d), 127.2 (s + d, 2C), 118.3 (d), 113.0 (d), 111.7 (d), 62.3 (d), 54.2 (t), 49.6 (d), 35.2 (t) ppm. ESI-MS m/z (ES^+) 474.06 [$(M+1)^+$, 100]. Anal. C18H17Cl2N3O6S (C, H, N).

4.2.10. (2R,3S)-3-(benzyloxy)-1-((3,4-dichlorophenyl)sulfonyl)-N-hydroxypyrrolidine-2-carboxamide (**38**)

Compound **35** (100 mg, 0.22 mmol) was dissolved in anhydrous MeOH in an oven-dried, sealed microwave vial under N_2 atmosphere, and a preformed solution of hydroxylamine hydrochloride (46 mg, 0.66 mmol) and KOH (74 mg, 1.32 mmol) in MeOH was added. The vessel was heated for 1 h at 80 °C in a microwave reactor. The mixture was acidified with 1 M HCl to pH 3. The resulting solution was evaporated, and then diluted with H_2O. Aqueous solution was extracted with EtOAc, and the organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by flash chromatography (silica gel, et. pet. ether/EtOAc 1:1, R_f 0.4), affording **38** as colorless oil (13 mg, 14% yield). $[\alpha]^{24}_D$ −86.0 (c = 0.65, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.58 (br, 1H), 7.89 (d, J = 1.9 Hz, 1H), 7.57 (dd, J = 1.9, 8.2 Hz, 1H), 7.30 (d, J = 8.2 Hz, 1H), 7.21–7.19 (m, 3H), 6.87–6.84 (m, 2H), 4.26–4.20 (m, 2H), 3.64 (m, 1H), 3.26–3.17 (m, 1H), 1.98–1.94 (m, 2H), 0.88–0.79 (m, 2H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ 166.0 (s), 138.1 (s), 136.3 (s), 134.4 (s), 133.5 (s), 130.7 (d), 129.5 (d), 128.0 (d), 127.5 (d), 126.6 (d), 80.7 (d), 70.3 (t), 66.1 (d), 47.7 (t), 29.9 (t) ppm. ESI-MSMS m/z (ES^+) 445.44 [$(M+1)^+$, 20.37], 385.38 [$(M\text{-}CONHOH)^+$, 100]. Anal. C18H18Cl2N2O5S (C, H, N).

4.2.11. (2R,3S)-3-(benzyloxy)-1-((4-chlorophenyl)sulfonyl)-N-hydroxypyrrolidine-2-carboxamide (**39**)

To a solution of **36** (122 mg, 0.29 mmol) in MeOH (1 mL) was added dropwise a preformed solution of NH_2OK (0.87 mmol) in MeOH (522 μL). The mixture was stirred at room temperature for 48 h. The reaction mixture was then acidified with 1 M HCl to pH 3 and extracted with EtOAc. The organic layers were dried over anhydrous Na_2SO_4, filtered and evaporated. The crude product was purified by automated flash chromatography (pet. ether/EtOAc 1:2), to obtain compound **39** as white solid (35 mg, 29% yield). $[\alpha]^{28}_D$ −80.8 (c = 1.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.76 (d, J = 8.5 Hz, 2H), 7.33 (d, J = 7.8 Hz, 2H), 7.27 (m, 3H), 6.90 (m, 2H), 4.25 (m, 2H), 4.24 (s, 2H), 3.69 (m, 1H), 3.25 (m, 1H), 1.99 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 179.9 (s), 140.2 (s), 137.8 (s), 134.4 (s), 129.6 (d, 2C), 128.5 (d), 127.9 (d), 127.3 (d), 81.4 (d), 70.7 (t), 66.5 (d), 48.2 (t), 30.4 (t) ppm. ESI-MSMS m/z (ES^+) 433.00 [$(M + Na)^+$, 20.80], 244.00 (100). Anal. C18H19ClN2O5S (C, H, N).

4.2.12. (2R,3S)-1-((4-chlorophenyl)sulfonyl)-3-(hexyloxy)-N-hydroxypyrrolidine-2-carboxamide (**40**)

To a solution of **37** (102 mg, 0.24 mmol) in MeOH (1 mL) was added dropwise a preformed solution of NH_2OK (0.72 mmol) in MeOH (600 μL). The resulting mixture was stirred at room temperature for 48 h. Then, it was acidified with 1 M HCl (pH 3) and extracted with EtOAc. The organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure, to give orange oil. The crude product was purified by flash cromatography (silica gel, pet. ether/EtOAc 1:2, R_f 0.42), giving compound **40** as a yellow oil (12 mg, 12% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.75 (d, J = 8.5 Hz, 2H), 7.43 (d, J = 8.5 Hz, 2H), 4.04 (s, 1H), 3.60 (1H), 3.18 (m, 1H), 3.02 (m, 2H), 1.86 (m, 2H), 1.22–0.95 (m, 8H), 0.80 (t, J = 6.9 Hz, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 166.6 (s), 140.2 (s), 129.5 (d), 129.4 (d), 81.3 (d), 68.9 (t), 66.1(d), 48.0 (t), 31.5 (t), 30.0 (t), 29.3 (t), 25.4 (t), 22.5 (t), 14.0 (q) ppm. ESI-MSMS m/z (ES^+) 404.42 (M^+, 10.99), 175.05 (100). Anal. C17H25ClN2O5S (C, H, N).

4.3. Enzyme inhibition assay

The inhibition of LF activity by final compounds was measured by a spectrophotometric assay. It was conducted using anthrax lethal factor protease substrate II (Ac–Gly–Tyr–ßAla–Arg–Arg–Arg–Arg–Arg–Arg–Arg–Arg–Val–Leu–Arg–pNA) as peptide substrate. Peptide bond cleavage was measured monitoring the release of *p*-nitroaniline as an increase in absorbance at 405 nm wavelength. Each reaction contained the colorimetric substrate (10 μM), the inhibitors at increasing concentrations, and anthrax lethal factor (10 nM) in 250 mM Na_2HPO_4, 150 mM NaCl, pH = 7.4 at 25 °C. After LF addition to the reaction mixture, decrease of absorbance at 405 nm was followed for 10 min. The IC_{50} values were obtained by dose–response measurements using the inhibitor in a range of concentrations: 500 nM, 1 μM, 2.5 μM, 5 μM, 10 μM. All the experiments were repeated twice. The IC_{50} values were determined by fitting binding inhibition data by non-linear regression using GraphPad Prism 4.0 Software Package (GraphPad Prism, San Diego, CA).

4.4. Tridimensional structure alignment

The Swiss PDB viewer (SPDBV) program (4.0.1 version, Swiss Institute of Bioinformatics) was used to superimpose three dimensional (3D) structures of anthrax lethal factor in complex with L915 (PDB code: 1YQY), and LF in complex with BI-MFM3 (PDB code: 1ZXV) [32,33]. The two PDB structures were superimposed using the “magic fit” option. Then, using the “improve fit” option implemented in SPDBV, iterations were performed until convergence of RMS and maintaining the number of matching residues as high as possible. Structural alignment was generated after protein superimposition. The residues of the superimposed protein that were spatially close to residues of the static one were aligned. Appropriate gaps were inserted in the sequences to indicate a lack of structural correspondence. The quality of the superimposition was evaluated by calculating the RMS at each residue.

4.5. Docking calculation

Docking analysis was performed using the GOLD (Genetic Optimization for Ligand Docking) software package 5.1 version (CCDC, Cambridge, UK) [34]. The 3D structure of the ligand was generated using SPARTAN version 5.147 running on an SGI IRIX 6.5 workstation, and the AM1 semiempirical method [35] was used to optimize the global minimum conformer. The coordinates of LF enzyme were retrieved from the Protein Data Bank (PDB code:1YQY). The ligand-protein complex was unmerged to achieve free enzyme structure, water molecules were removed and hydrogen atoms were added to the enzyme. The binding site was defined as all residues of the protein within 10 Å from the γ-carbon of His686. Goldscore was chosen as the fitness function, and standard default settings were used in all the calculations. The zinc metal ion was set up in a tetrahedral coordination. For each of the 50 independent genetic algorithm runs, a maximum of 10e6 genetic operations were carried out, using the default operator weights and a population size of 100 chromosomes. Results differing by less than 1.5 Å in ligand-all atom RMSD, were clustered together. The analysis of binding mode of the docked conformations were carried out using PyMol software v.0.99 [36].

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