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# Identification of *Trypanosoma brucei* leucyl-tRNA synthetase inhibitors by pharmacophore- and docking-based virtual screening and synthesis

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ABSTRACT

Human African trypanosomiasis (HAT), caused by the protozoan parasite *Trypanosoma brucei*, is a neglected fatal disease. Leucyl-tRNA synthetase (LeuRS), which has been successfully applied in the development of antifungal agent, represents a potential antiprotozoal drug target. In this study, a 3D model of *T. brucei* LeuRS (*Tb*LeuRS) synthetic active site was constructed and subjected to virtual screening using a combination of pharmacophore- and docking-based methods. A new 2-pyrrolinone scaffold was discovered and the structure–activity relationship (SAR) studies aided by the docking model and organic synthesis were carried out. Compounds with various substituents on $R^1$, $R^2$ and $R^3$ were synthesized and their SAR was discussed.



## 1. Introduction

The protozoan parasite *Trypanosoma brucei* causes human African trypanosomiasis (HAT) which is also known as sleeping sickness. It is a neglected fatal disease and continues to pose a major threat to 60 million people in 36 countries in sub-Saharan Africa.[1,2] Nevertheless, only a limited number of clinically useful drugs are available for HAT.[3] They are pentamidine (discovered in 1941), suramin (discovered in 1921), melarsoprol (discovered in 1949) and eflornithine (registered in 1990). All of the four licensed compounds are generally unsatisfactory due to a combination of low efficacy, severe side effects, and difficulty in administration. Thus, new therapeutics for the treatment of HAT are urgently needed.

Aminoacyl-tRNA synthetases (aaRSs) play a central role in the process of protein synthesis. They are responsible for catalyzing the attachment of the correct amino acid to its cognate tRNA by a two-step aminoacylation reaction.[4] The first step is the formation of an aminoacyl-adenylate (aa-AMP), as an activated intermediate, from amino acid and ATP. In the second step, the activated amino acid is transferred from aa-AMP to the 3′-terminal adenosine of tRNA to form aminoacyl-tRNA (aa-tRNA). The aaRSs are divided into two classes (class I and class II) based on mutually exclusive sets of sequence motifs that reflect distinct active site topologies: the class I synthetase active site contains a Rossmann-fold domain while the class II contains an antiparallel β-fold domain.[5] Leucyl-tRNA synthetase (LeuRS) belongs to class I and contains two conserved characteristic motifs, with the consensus sequences of His-Ile-Gly-His (HIGH) and Lys-Met-Ser-Lys-Ser (KMSKS).[4] LeuRS contains a synthetic domain and a connective polypeptide 1 (CP1) editing domain. The synthetic domain is in charge of the aminoacylation reaction, whereas, the editing domain takes responsibility for hydrolyzing mischarged tRNA to ensure the high fidelity required by translation.[6,7]

LeuRS is a clinically validated drug target that has been successfully used for the development of antifungal agent.[8,9] We recently reported a class of inhibitors that target the editing active site of *T. brucei* LeuRS (*Tb*LeuRS) as novel antitrypanosomal agents.[10] There are also some LeuRS synthetic active site inhibitors, but none is for *T. brucei*.[11–15] Despite of the fact that *Tb*LeuRS could serve as a valuable therapeutic target for the treatment of trypanosomiasis,[16–18] inhibitors targeting at its synthetic active site have never been reported to date. In the present study, we will discuss the first discovery of *Tb*LeuRS synthetic active site inhibitors.

In silico screening has been widely applied in the discovery of lead compounds in the initial stage of drug development,[19,20] and it has been successfully used in the identification of aaRS inhibitors as demonstrated by the discovery of methionyl-tRNA synthetase (MetRS)[21–23] and tryptophanyl-tRNA synthetase (TrpRS)[24] inhibitors whereas either

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pharmacophore-based or docking-based virtual screening was employed. In this study, we combined both pharmacophore- and docking-based approaches to discover *Tb*LeuRS inhibitors. The three dimensional (3D) structure of *Tb*LeuRS was modeled using the known *Pyrococcus horikoshii* LeuRS (*Ph*LeuRS) structure as a template and was screened against SPECS database which consists of approximately 197,000 commercially available compounds. A novel 2-pyrrolinone scaffold represented by compounds **1** and **8** (Table 1) were discovered. Base on the docking model of compound **8** in *Tb*LeuRS, compounds with various substituents on $R^1$, $R^2$ and $R^3$ were synthesized and the structure–activity relationship was analyzed. In general, the experimental observations corroborated the docking model, which showed the $R^2$ phenyl inserted into a new hydrophobic pocket that was not occupied by the endogenous substrate, and the $R^3$ indolyl was essential due to its interaction with the leucine-recognition pocket.

## 2. Results and discussion

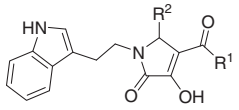
### 2.1. Construction of the 3D structure of *T. brucei* LeuRS synthetic active site

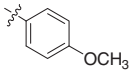
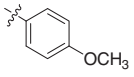
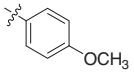
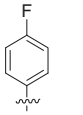
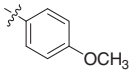
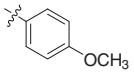
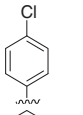
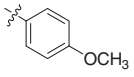
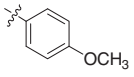
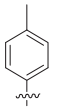
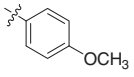
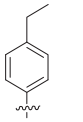
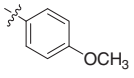
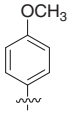
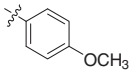
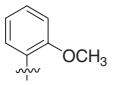
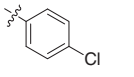
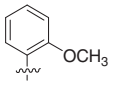
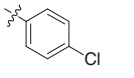
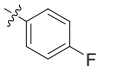
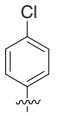
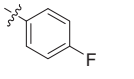
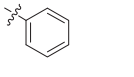
The currently known LeuRS X-ray crystal structures containing the synthetic domain include *P. horikoshii* and *Thermus thermophilus* LeuRS (*Ph*LeuRS and *Tt*LeuRS) structures in the Protein Data Bank (PDB).[25,26] Both *Ph*LeuRS and *Tb*LeuRS are the eukaryal/archaeal subtype, while the *Tt*LeuRS is the bacterial subtype. Thus, the *Ph*LeuRS structure (PDB ID: 1WKB, 2.05 Å, the active sites of *Ph*LeuRS and *Tb*LeuRS share 70% sequence identities)[25] was chosen as the template in the construction of the *Tb*LeuRS structure. Due to the absence of a ligand in *Ph*LeuRS (1WKB), the coordinates of Leu-AMS, which is a stable analog of endogenous substrate Leu-AMP, from *Tt*LeuRS (PDB ID: 1H3N, 2.00 Å, the active sites of *Tt*LeuRS and *Tb*LeuRS share 36% sequence identities)[26] was used to generate a hypothetical ligand space in *Ph*LeuRS and *Tb*LeuRS.

LeuRS is a large protein consisting of about 1000 amino acid residues. As shown by the sequence alignment in Figure 1, the homology is about 30% between the full length *Tb*LeuRS and *Ph*LeuRS. However, the synthetic active sites within 5 Å of the Leu-AMS ligand share about 70% sequence identities (red shaded, Fig. 1), and the characteristic HIGH and KMSKS motifs of class I aaRS[4] are also conserved (black stars, Fig. 1), which made it possible to construct a reliable *Tb*LeuRS active site for the purpose of virtual screening. Therefore, although the synthetic domain consists of four discontinuous fragments (orange boxes, Fig. 1) separated by several domains including the CP1 editing domain (green residues, Fig. 1) and presented a challenge for homology modeling, the highly conserved active site provided an opportunity to simulate a practical *Tb*LeuRS synthetic active site by mutation method. The core synthetic active site of *Tb*LeuRS was constructed by mutating the active site residues within 8 Å of Leu-AMS in *Ph*LeuRS to their corresponding *Tb*LeuRS residues. The ligand–protein interactions involved in the following pharmacophore and docking models were found to be well within 5 Å of Leu-AMS, and the layer between 5 and 8 Å served as a buffer environment for the inner core sphere.

LeuRS proteins from different species bind to the same leucine, ATP and leucyl-adenylate substrates, and catalyze the same aminoacylation reaction. A highly conserved hydrophobic pocket in almost all LeuRSs accommodates the substrate leucine side chain.[26] A conserved Asp residue makes hydrogen bond to the α-amino group of leucine, and the key conserved Glu/Asp and Gln residues make hydrogen bond interactions with the ribose and adenine of the substrate leucyl-adenylate. The class I characteristic motifs, HIGH and KMSKS, are also highly conserved (Fig. 1). Thus, the active sites of all LeuRSs are highly conserved as exemplified by the excellent superposition of *Tt*LeuRS and *Ph*LeuRS active sites[25] even although they belong to bacterial and eukaryal/archaeal subtypes, respectively. *Tb*LeuRS and *Ph*LeuRS, both belonging to the eukaryal/archaeal subtype, share 70% sequence identities for

**Table 1**
Inhibitory effect of hit compound **1** from virtual screening and its related analogs from SPECS against *Tb*LeuRS (average error within 0.05 μM)




| Compd | $R^1$ | $R^2$ | *Tb*LeuRS $IC_{50}$ (μM) |
|---|---|---|---|
| **1** |  |  | 170.3 |
| **2** |  |  | 268.6 |
| **3** |  |  | 64.7 |
| **4** |  |  | 125.3 |
| **5** |  |  | 86.9 |
| **6** |  |  | 260.3 |
| **7** |  |  | 117.3 |
| **8** |  |  | 71.4 |
| **9** |  |  | 333.5 |
| **10** |  |  | 189.8 |
| **11** |  |  | 107.0 |
| **12** |  |  | 588.5 |
| **13** |  |  | >1000 |
| **14** |  |  | >1000 |
| **15** |  |  | >1000 |

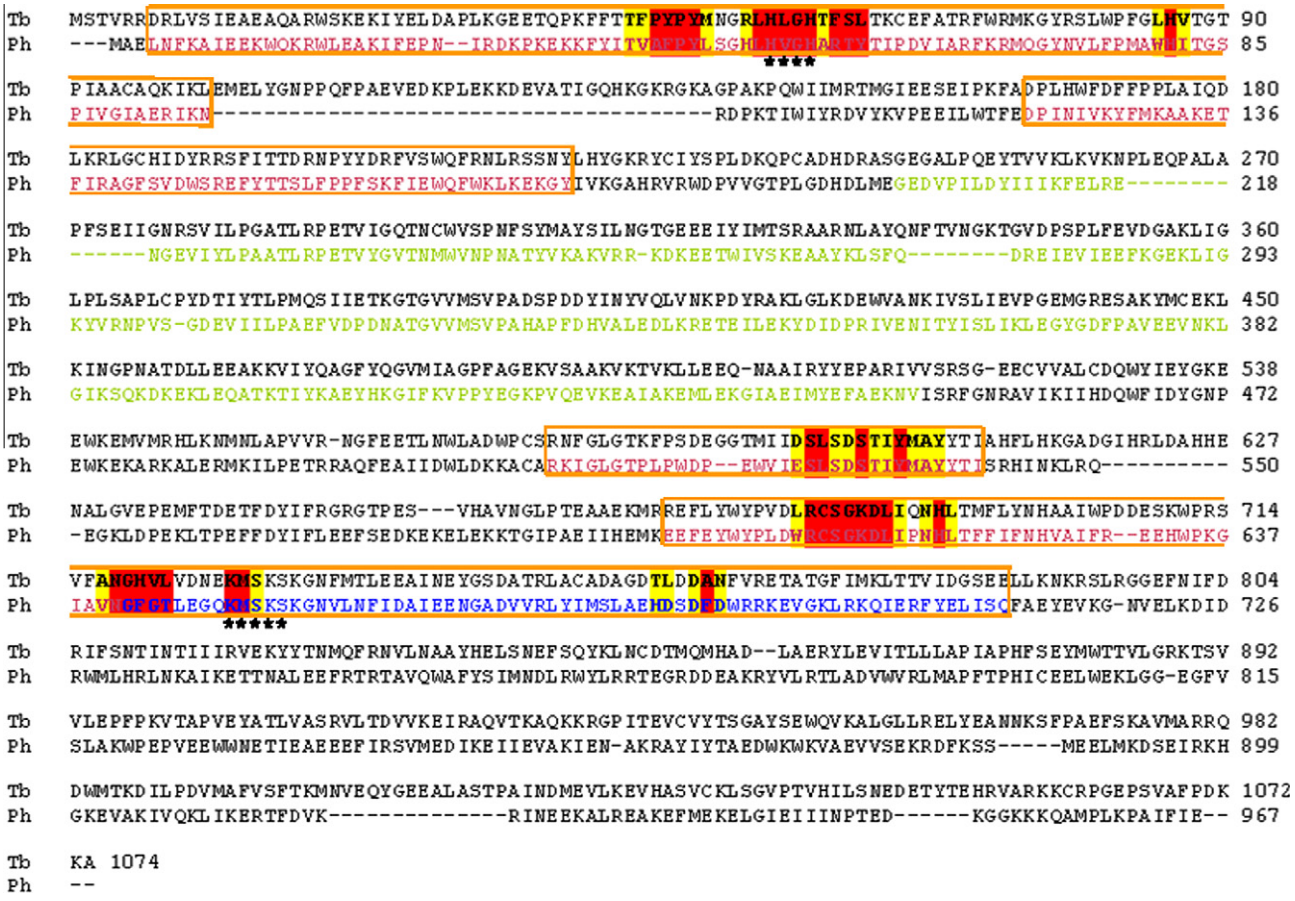


**Figure 1.** Sequence alignment of *Tb*LeuRS and *Ph*LeuRS. The *Ph*LeuRS residues in salmon font are Rossmann-fold domain, in green font are CP1 editing domain, and in blue font are SC-fold domain. Residues in orange box are the catalytic domain of LeuRS. The red shaded residues are those within 5 Å of Leu-AMS ligand, yellow shaded are within 5–8 Å. The HIGH and KMSKS motifs were labeled by black stars.

the active sites. Therefore, the *Tb*LeuRS synthetic active site constructed by mutation method from *Ph*LeuRS should serve as a reasonable model for the following virtual screening.

Although the synthetic active sites of *Tb*LeuRS and *Ph*LeuRS share high sequence identities and the *Tb*LeuRS active site was built based on *Ph*LeuRS, they are distinct from each other. As shown in Fig. 1 and 10 of the 33 synthetic active site residues (red shaded) in *Tb*LeuRS and *Ph*LeuRS are different. Most of the different residue pairs own distinct properties (e.g., F60 (*Tb*LeuRS)/R55(*Ph*LeuRS), L62/Y57, H720/F643), which makes it feasible to design inhibitors specific for the synthetic active site of *Tb*LeuRS.

## 2.2. Inhibitor identification by virtual screening

A combination of pharmacophore- and docking-based approaches was employed in the virtual screening to identify inhibitors against *Tb*LeuRS synthetic active site.

First, based on the analysis of the characteristic interaction patterns found between endogenous substrate analog Leu-AMS and *Tb*LeuRS,[8,26,27] pharmacophores I-IV (Figs. 2a and c and S1) were generated. These pharmacophore models all incorporated three common features that are intrinsic to the substrate–protein interactions (Figs. 2 and S1): the hydrophobic site (HPS) feature at the isobutyl group; the hydrogen bond donor (HBD) feature at the amino group of leucyl moiety; the hydrogen bond donor (HBD) feature at the OH group of ribose which interacts with a conserved Asp residue. The four pharmacophore models varied in the hydrogen bond patterns at the purine group as shown in Figure 2 and S1.

A set of 20 molecules comprising substrate analog Leu-AMS, 10 known LeuRS inhibitors,[11–15] and nine random compounds from SPECS database were used to evaluate the pharmacophore models. Pharmacophores I and II returned 9 and 10 hits, respectively, which is proved to be superior over III and IV which returned 5 and 4 hits, respectively. Thus, pharmacophores I and II were selected to screen the SPECS database using Catalyst and resulted in 302 and 1282 hits, respectively. Subsequently, the hits were submitted to docking evaluation using Glide. Finally, the binding poses of the top 600 molecules were manually inspected using the following criteria: the binding mode should be consistent with Leu-AMS; the HPS feature of pharmacophore models should be matched; more than two hydrogen bonds should be found; diversity of scaffolds should be considered. The selected 26 compounds were tested against *Tb*LeuRS, and 2-pyrrolinone **1** was found to be active with an $IC_{50}$ of 170.3 μM (Table 1). Compound **1** matched well with pharmacophore I (Fig. 2b). The indolyl group acts as the hydrophobic feature corresponding to the isobutyl group of Leu-AMS. The NH group of indolyl and the OH group of pyrrolinone act as the hydrogen bond donor features in the same manners as the amino and the OH groups in the leucyl and ribose moieties of Leu-AMS, respectively. The methoxy oxygen acts as the hydrogen bond acceptor feature in place of the adenine nitrogen of Leu-AMS.

## 2.3. Initial structure–activity relationship

To investigate the initial structure–activity relationship, more 2-pyrrolinone compounds with related structures were selected from

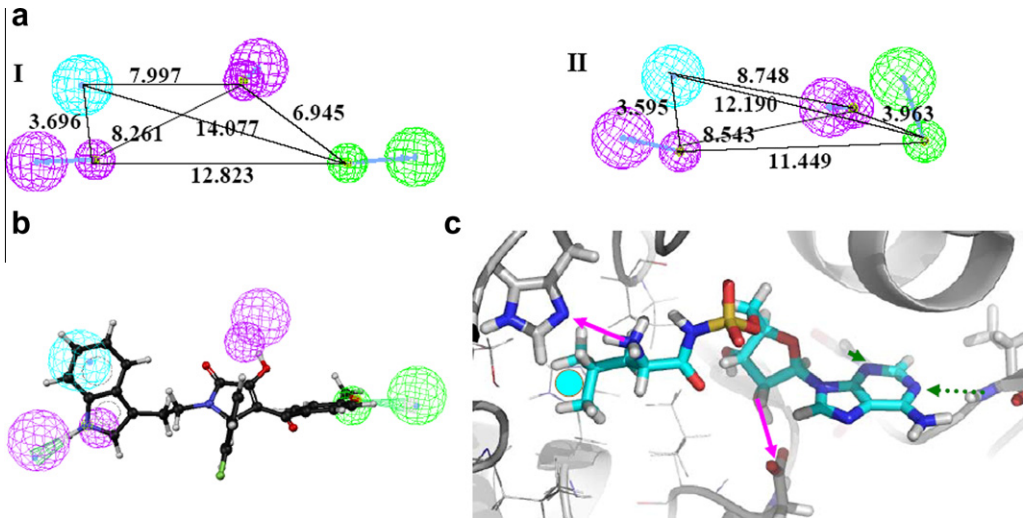



**Figure 2.** (a) Pharmacophores I and II: light blue, HPS (hydrophobic site); violet, HBD (hydrogen bond donor); green, HBA (hydrogen bond acceptor). Distances are shown in Å. (b) Overlap of compound **1** with pharmacophore I. (c) Leu-AMS docked in *Tb*LeuRS active site with pharmacophore features labeled in the same color as (a). The HBA feature of pharmacophore I is show in green dashed arrow while that of pharmacophore II is shown in green solid arrow.

the SPECS database and tested against *Tb*LeuRS (Table 1). A number of compounds with $IC_{50}$ values below 100 μM were identified and an initial structure–activity relationship was established.

The effect of substitutions on the $R^2$ phenyl group was first investigated (Table 1). The *para*-halogen substitutions gave more favorable activity than *meta*- or *ortho*-substitution as demonstrated by compounds **1**–**3** and **4**–**5**. The *para*-substitution with halogen (**3** and **5**) or alkyl (**7** and **8**) gave improved potency as compared to unsubstituted compound **6**. Ethyl-gave better potency than methyl (**8** and **7**). However, polar substituents at *para*- or *meta*-position led to diminished activity as shown by compound **9** and **12**. These observations suggest the existence of a hydrophobic binding pocket near the *para*-position of $R^2$ phenyl. Next, the effect of substitutions on $R^1$ was considered. As shown by the improved potency of compound **11** compared to **10**, chloro-substitution gave improved activity. However, fluoro-substitution did not have such effect, as shown by the abolished activity of compounds **13** and **14**. Removal of the methoxy group in **1** also resulted in abolished activity (**15**).

To facilitate the understanding of the interaction between 2-pyrrolinones and the synthetic active site of *Tb*LeuRS, 2-pyrrolinone **8** was docked into *Tb*LeuRS using Glide. As shown in Figure 3, compound **8** assumed a similar binding pose to the endogenous substrate analog Leu-AMS. The indolyl group partially inserted into a hydrophobic pocket composed by Pro46, Tyr47, Ser599, Leu596, His692, and Leu688, and formed favorable hydrophobic interactions. The indolyl NH group formed a hydrogen bond with the backbone carbonyl of Tyr47. The exocyclic tautomeric hydroxyl formed a

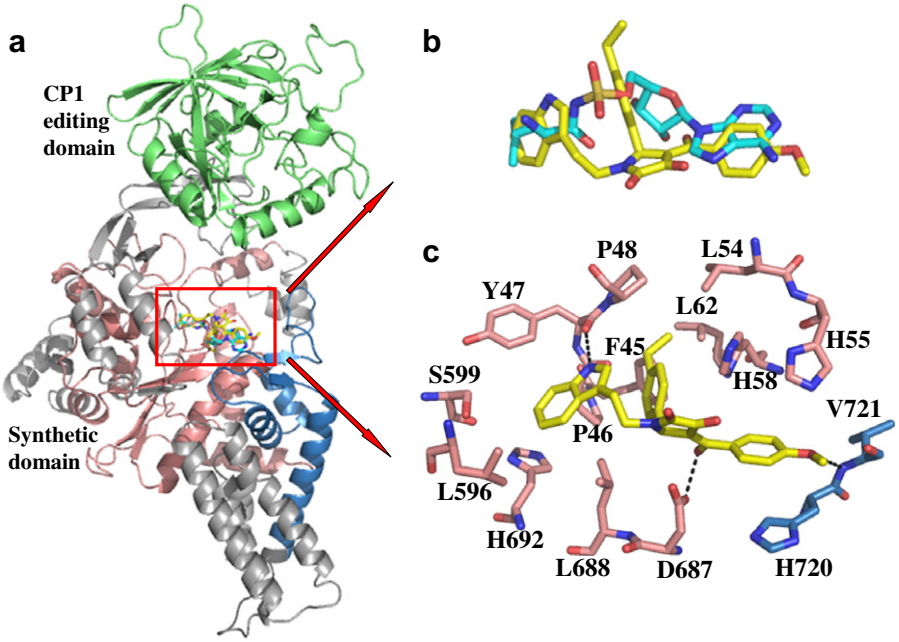
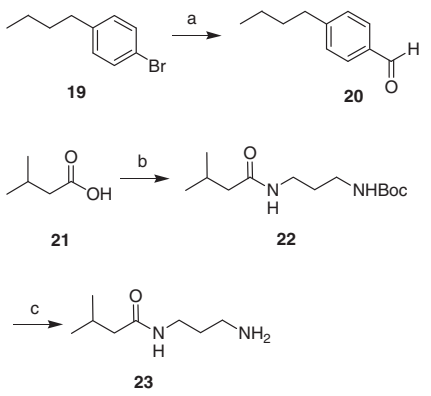
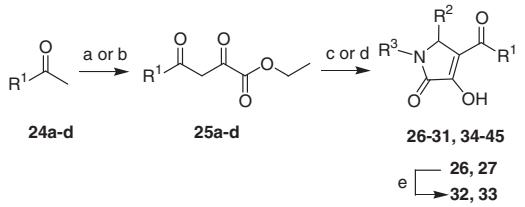



**Figure 3.** Binding mode of compound **8** in *Tb*LeuRS. (a) Illustration of full length LeuRS structure containing CP1 editing domain and synthetic domain. Salmon: Rossmann-fold; blue: SC-fold; green: CP1 editing domain. (b) Superposition of compound **8** (yellow) and Leu-AMS (cyan) in *Tb*LeuRS synthetic active site. (c) Main interactions between compound **8** and *Tb*LeuRS synthetic active site. Dashed lines represent hydrogen bonds. This figure was generated in PyMoL.




**Scheme 1.** Synthesis of aldehyde and amine intermediates. Reagents and conditions: (a) (i) *n*-BuLi, THF, −78 °C; (ii) DMF, THF, −78 °C to rt (b) *tert*-butyl 3-aminopropylcarbamate, EDCI, DMAP, DCM, 0 °C to rt; (c) HCl, DCM.




**24a, 25a**: $R^1$ = 4-$OCH_3$-Ph
**24b, 25b**: $R^1$ = 4-Cl-Ph
**24c, 25c**: $R^1$ = 2, 4-difluorophenyl
**24d, 25d**: $R^1$ = 1-napthyl

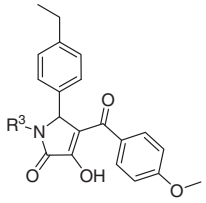
**26**: $R^1$ = 4-$OCH_3$-Ph, $R^2$ = 4-Et-Ph, $R^3$ = 3-(*N*-Boc)$NH_2$-Pr
**27**: $R^1$ = 4-$OCH_3$-Ph, $R^2$ = 4-Et-Ph, $R^3$ = 4-(*N*-Boc-$NH_2CH_2$)-Ph
**28**: $R^1$ = 4-$OCH_3$-Ph, $R^2$ = 4-Et-Ph, $R^3$ = 3-(*N*-isovaleryl)aminopropyl
**29**: $R^1$ = 4-$OCH_3$-Ph, $R^2$ = 4-Et-Ph, $R^3$ = (benzo[1, 3]dioxol-5-yl)methyl
**30**: $R^1$ = 4-$OCH_3$-Ph, $R^2$ = 4-Et-Ph, $R^3$ = 4-COOH-Bn
**31**: $R^1$ = 4-$OCH_3$-Ph, $R^2$ = 4-Et-Ph, $R^3$ = 3-(imidazol-1-yl)propyl
**32**: $R^1$ = 4-$OCH_3$-Ph, $R^2$ = 4-Et-Ph, $R^3$ = 3-$NH_2$-Pr
**33**: $R^1$ = 4-$OCH_3$-Ph, $R^2$ = 4-Et-Ph, $R^3$ = 4-($NH_2CH_2$)-Ph

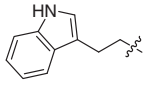
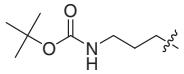
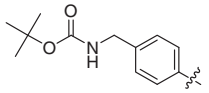
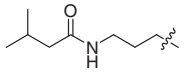
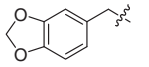
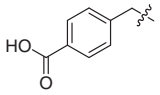
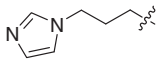
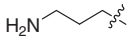
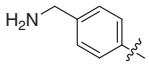
**34**: $R^1$ = 4-$OCH_3$-Ph, $R^2$ = 4-$CF_3$-Ph, $R^3$ = 2-(indol-3-yl)ethyl
**35**: $R^1$ = 4-$OCH_3$-Ph, $R^2$ = 4-*i*-Pr-Ph, $R^3$ = 2-(indol-3-yl)ethyl
**36**: $R^1$ = 4-$OCH_3$-Ph, $R^2$ = 4-*t*-Bu-Ph, $R^3$ = 2-(indol-3-yl)ethyl
**37**: $R^1$ = 4-$OCH_3$-Ph, $R^2$ = 4-*n*-Bu-Ph, $R^3$ = 2-(indol-3-yl)ethyl
**38**: $R^1$ = 4-$OCH_3$-Ph, $R^2$ = 4-*i*-Bu-Ph, $R^3$ = 2-(indol-3-yl)ethy
**39**: $R^1$ = 4-$OCH_3$-Ph, $R^2$ = biphenyl, $R^3$ = 2-(indol-3-yl)ethyl
**40**: $R^1$ = 4-$OCH_3$-Ph, $R^2$ = napth-2-yl, $R^3$ = 2-(indol-3-yl)ethyl
**41**: $R^1$ = 4-$OCH_3$-Ph, $R^2$ = 2-Br-4-F-Ph, $R^3$ = 2-(indol-3-yl)ethyl
**42**: $R^1$ = 4-$OCH_3$-Ph, $R^2$ = pentyl, $R^3$ = 2-(indol-3-yl)ethyl
**43**: $R^1$ = 4-Cl-Ph, $R^2$ = 4-*i*-Bu-Ph, $R^3$ = 2-(indol-3-yl)ethyl
**44**: $R^1$ = 2,4-difluorophenyl, $R^2$ = 4-*i*-Bu-Ph, $R^3$ = 2-(indol-3-yl)ethyl
**45**: $R^1$ = napth-1-yl, $R^2$ = 4-Et-Ph, $R^3$ = 2-(indol-3-yl)ethyl

**Scheme 2.** Synthesis of 2-pyrrolinone derivatives **26**–**45**. Reagents and conditions: (a) diethyl oxalate, LiHMDS, $Et_2O$, −78 °C to rt, then 1 M HCl (for compd **25a**); (b) diethyl oxalate, NaH, toluene, −78 °C to rt(for compds **25b**–**d**); (c) $R^2CHO$, $R^3NH_2$, toluene or ethanol, DIPEA, reflux (for compds **26**, **28**, **38** and **45**); (d) $R^2CHO$, $R^3NH_2$, toluene or EtOH, reflux (for compds **27**, **29**–**31**, **34**–**37**, **39**–**44**); (e) HCl, DCM.

hydrogen bond with the highly conserved Asp687. The methoxyphenyl group was sandwiched by His55, His58, His720, and formed favorable π–π interactions. The methoxy oxygen formed a hydrogen bond with the backbone NH of Val721. It is worth mentioning that the docking model indeed showed the $R^2$ phenyl inserted into a new hydrophobic pocket consisting of Phe45, Pro48, Leu54 and Leu62, which was not occupied by Leu-AMS. These observations corroborate the initial structure–activity relationship discussed above and will aid the design of compounds with improved potency against *Tb*LeuRS.

**Table 2**
Inhibitory effect of synthesized compounds with various $R^3$ groups against *Tb*LeuRS (average error within 0.05 μM)




| Compd | $R^3$ | *Tb*LeuRS $IC_{50}$ (μM) |
|---|---|---|
| **8** |  | 71.4 |
| **26** |  | 269.0 |
| **27** |  | 101.9 |
| **28** |  | >1000 |
| **29** |  | 173.5 |
| **30** |  | 656.3 |
| **31** |  | 716.1 |
| **32** |  | >1000 |
| **33** |  | >1000 |

## 2.4. Studies of structure–activity relationship via new compound design and synthesis

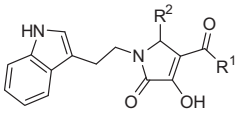
### 2.4.1. Chemistry

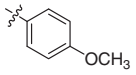
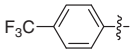
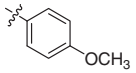
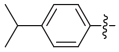
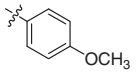
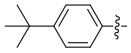
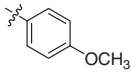
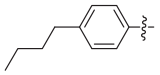
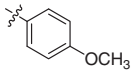
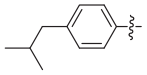
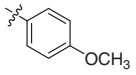
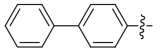
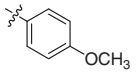
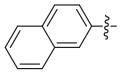
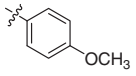
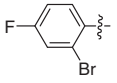
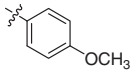
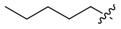
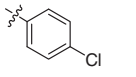
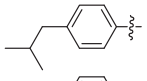
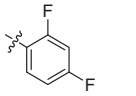
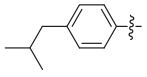
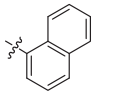
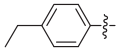
The aldehydes and amines used in the synthesis of 2-pyrrolinones were either purchased or prepared as exemplified by the synthesis of aldehyde **20** and amine **23** (Scheme 1). Treatment of 1-bromo-4-butylbenzene **19** with *n*-BuLi at −78 °C followed by reaction with DMF gave aldehyde **20**.[28] Coupling of 3-methylbutyric acid **21** with *tert*-butyl 3-aminopropylcarbamate in the presence of EDCI and DMAP provided N-Boc protected intermediate **22** which was converted to amine **23** after deprotection by HCl. The synthesis of 2-pyrrolinones was summarized in Scheme 2. First, diketo esters **25a**–**d** were obtained via Claisen condensation of ketones **24a**–**d** with diethyl oxalate in the presence of LiHMDS or NaH.[29–31] The subsequent three-component condensation reaction by refluxing diketo esters with appropriate aldehydes and amines gave 2-pyrrolinones **26**–**31** and **34**–**45**, while the presence of DIPEA as a catalyst was needed in the preparation of **26**, **28**, **38** and **45** (Scheme 2).[32–35] Removal of the terminal N-Boc protection of **26** and **27** provided **32** and **33**, respectively.

### 2.4.2. Structure–activity relationship

First, the effect of $R^3$ group on *Tb*LeuRS inhibitory activity was explored (Table 2). Replacement of the indolyl group in compound

**Table 3**
Inhibitory effect of synthesized compounds with various $R^1$ and $R^2$ groups against *Tb*LeuRS (average error within 0.05 μM)




| Compd | $R^1$ | $R^2$ | *Tb*LeuRS $IC_{50}$ (μM) |
|---|---|---|---|
| **34** |  |  | 68.1 |
| **35** |  |  | 90.5 |
| **36** |  |  | 94.5 |
| **37** |  |  | 43.9 |
| **38** |  |  | 31.9 |
| **39** |  |  | 43.1 |
| **40** |  |  | 85.5 |
| **41** |  |  | 109.6 |
| **42** |  |  | 110.8 |
| **43** |  |  | 41.0 |
| **44** |  |  | 175.4 |
| **45** |  |  | 80.0 |

**8** with either chain structures or different heterocycles with nonpolar or polar moieties all resulted in diminished activity ( **26 and 28–33**) except for compound **27** which showed a moderately decreased activity. It suggests that the interaction of the indolyl group with the hydrophobic pocket proposed by the docking model (Fig. 3) may be significant in the binding of 2-pyrrolinones to *Tb*LeuRS.

Next, the effect of $R^2$ group on the *Tb*LeuRS inhibitory activity was explored (Table 3). Since the docking model showed the $R^2$ group inserted into a new hydrophobic pocket (Fig. 3), various hydrophobic substituents including *p*-alkylphenyl (**34–38**), biphenyl (**39**), naphthyl (**40**), halogenated phenyl (**41**), and aliphatic chain (**42**) were synthesized. Compound **34** with *p*-$CF_3$-phenyl group showed comparable inhibitory activity to compound **8** with *p*-ethyl-phenyl. When the *p*-alkyl group became butyl or *iso*-butyl group, the inhibitory activity showed a significant improvement as demonstrated by compounds **37** and **38** ($IC_{50}$ = 43.9 and 31.9 μM). This observation corroborates the occupation of the new hydrophobic pocket by $R^2$ in the docking model. The biphenyl compound **39** also gave good inhibitory activity ($IC_{50}$ = 43.1 μM). Halogen substitutions (**41**) and replacement of the phenyl with a pentyl chain (**42**) both resulted in decreased activity. Considering the fact that the chlorine substitution for methoxy on $R^1$ phenyl group gave improved activity (compound **11** vs **10**), the chloride analog of compound **38** (**43**) was synthesized, but it showed a comparable $IC_{50}$ of 41.0 μM. The fluoride analog of compound **38** (**44**) showed decreased activity, while the naphthyl analog of compound **8** (**45**) showed comparable activity.

## 3. Conclusion

Human African trypanosomiasis, a fatal disease epidemic in sub-Saharan Africa, is in urgent need of new therapeutics, especially those with new mechanism of action. Here we reported the first discovery of inhibitors targeting the synthetic domain of *T. brucei* LeuRS. Virtual screening using a combination of pharmacophore- and docking-based approaches against a model of *Tb*LeuRS synthetic active site was carried out and a number of 2-pyrrolinones were discovered as *Tb*LeuRS inhibitors. Guided by the docking model of compound **8** in *Tb*LeuRS, 2-pyrrolinones with various substituents at $R^1$, $R^2$, and $R^3$ were designed and synthesized. The structure–activity relationship generally corroborated the docking model, which showed the $R^2$ phenyl explored a new hydrophobic pocket, and the $R^3$ indolyl is essential for the favorable interaction with the leucine-recognition pocket. This work provided a new approach and lead compounds for the exploration of *T. brucei* LeuRS as a novel therapeutic target for the discovery of new anti-parasitic agents.

## 4. Experimental section

### 4.1. Construction of the 3D structure of *Tb*LeuRS synthetic active site

The synthetic active site of *T. brucei* LeuRS was constructed based on the crystal structure of *P. horikoshii* LeuRS (1WKB).[25] The coordinates of ligand Leu-AMS from *T. thermophilus* LeuRS (1H3N)[26] was used to generate a hypothetical ligand space in *Ph*LeuRS and *Tb*LeuRS. The active site residues in *Ph*LeuRS within 8 Å of the Leu-AMS ligand were mutated to the corresponding residues of *Tb*LeuRS. Hydrogen atoms were added, bond orders were designed, and water molecules were deleted in the Protein Preparation Wizard work flow in the Maestro interface.[36] The added hydrogen atoms were optimized by H-bond Assignment with exhaustive sampling method. Finally, the structure was subjected to minimization in MacroModel[37] using OPLS_2005 force field.[38] The residues within 5 Å of ligand were set to be free, those between 5 and 8 Å were constrained by 200 kcal/(mol $*$ $A^2$) force, and those between 8 and 10 Å were frozen.

### 4.2. In silico screening

The crucial interaction patterns between *Tb*LeuRS and Leu-AMS were analyzed using LigandScout.[39] Four pharmacophore models, I-IV (Figs. 2 and S1), were generated and subsequently evaluated by a set of 20 molecules including substrate analog Leu-AMS, 10 known LeuRS inhibitors[11–15] (Table S1) and nine random compounds from SPECS database (~197,000 compounds, http://www.specs.net). The pharmacophores I and II were selected to screen against SPECS database using Catalyst with Best Flexible Search mode. Molecules that fit all the features of pharmacophore model I or II were retained as hits, and 302 and 1282 hits were returned, respectively.

Subsequently, all hits were prepared by LigPrep using OPLS_2005 force field[38] and the ionization states at pH 7.0 ± 2.0 were generated using Epik before they were docked to *Tb*LeuRS using Glide in SP

precision. The *Tb*LeuRS grid was generated in Receptor Grid Generation and the docking box was defined as a 10 Å * 10 Å * 10 Å bounding box with Leu-AMS at the center. The docking box well covered the complete active site.

### 4.3. Chemistry

NMR spectra were recorded on Bruker Avance III 400 MHz or Varian Mercury 300 Hz. Chemical shifts are expressed in parts per million ($\delta$) relative to residual solvents as internal reference ($CDCl_3$: 7.26; MeOD: 3.31; DMSO-$d_6$: 2.50). High resolution mass spectra were obtained on an Agilent 6530 Accurate Mass Q-TOF LC-MS. Column chromatography was performed using Huanghai silica gel (45–75 μm). Melting points were measured on a SGWX-4 melting point apparatus. The preparative HPLC was conducted on an Unimicro EasySep-1010 liquid chromatograph using a $C_{18}$ column (30 mm × 250 mm), 1 mL sample loop, a flow rate of 16 mL/min, and a gradient of 10% v/v MeOH in $H_2O$ (containing 0.1% v/v TFA) ($t$ = 0.0 min) to 100% MeOH ($t$ = 30.0 min). All reagents were commercially available if not indicated otherwise. Reactions were not optimized for maximum yields.

#### 4.3.1. 4-*n*-Butylbenzaldehyde (20)

To a solution of 4-bromo-4-butylbenzene **19** (4.0 g, 18.8 mmol) was added *n*-butyllithum (35 mL, 1.6 M in hexane, 56.3 mmol) over 30 min at −78 °C. After stirring at −78 °C for 2 h, dimethylformamide (3.7 mL, 56.3 mmol) in 10 mL dry tetrahydrofuran was added over 15 min. The mixture was warmed to room temperature gradually, poured into ice-water, and extracted by petroleum ether (100 mL × 4). The organic layers were combined, washed with 100 mL brine, dried over anhydrous sodium sulfate, and evaporated in vacuo. The residue was purified by column chromatography on silica gel using dichloromethane/petroleum ether (1/10, v/v) as eluent to give **20** (2.62 g, 86.1% yield) as a yellowish oil. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 9.97 (s, 1H), 7.79 (d, 2H, $J$ = 7.6 Hz), 7.33 (d, 2H, $J$ = 7.6 Hz), 2.69 (t, 2H, $J$ = 7.6 Hz), 1.62 (m, 2H), 1.36 (m, 2H) and 0.93 (t, 3H, $J$ = 7.2 Hz) ppm.

#### 4.3.2. *N*-(3-Aminopropyl)-3-methylbutyramide hydrochloride (23)

EDCI (220 mg, 2.3 mmol) and DMAP (6.6 mg, 0.054 mmol) in 5 mL dichloromethane was added dropwise to a mixture of 3-methylbutyric acid **21** (140 mg, 1.38 mmol) and *tert*-butyl 3-aminopropylcarbamate (200 mg, 1.15 mmol) in 4 mL dichloro methane at 0 °C under nitrogen atmosphere. The mixture was allowed to warm to room temperature and stirred overnight. The solution was washed by 1 M NaOH (2 mL), 1 M hydrochloric acid (2 mL), and saturated sodium bicarbonate solution (2 mL), dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate (1/1, v/v) as eluent to give *tert*-butyl 3-(3-methylbutyrylamino)propylcarbamate **22** (190 mg, 64.1% yield) as a white solid. $^1$H NMR: (300 MHz, $CDCl_3$): $\delta$ 6.18 (br s, 1H), 4.94 (br s, 1H), 3.28 (m, 2H), 3.16 (m, 2H), 2.04–2.26 (m, 3H), 1.56–1.65 (m, 2H), 1.44 (s, 9H) and 0.938 (d, 6H, $J$ = 6.0 Hz) ppm. Mp: 88–90 °C

After treatment of **22** (95 mg, 0.37 mmol) with dry HCl in dichloromethane, the solvent was evaporated to give compound **23** (68 mg, 94.7% yield) as a white solid. $^1$H NMR: (400 MHz, $D_2O$): $\delta$ 3.25(t, 2H, $J$ = 6.8 Hz), 2.96 (t, 2H, $J$ = 7.6 Hz), 2.07 (d, 2H, $J$ = 7.2 Hz), 1.94 (m, 1H), 1.83 (m, 2H) and 0.87 (d, 6H, $J$ = 6.8 Hz).

#### 4.3.3. (*Z*)-Ethyl 2-hydroxy-4-(4-methoxyphenyl)-4-oxo-2-butenoate (25a)

To a solution of LiHMDS (41.5 mL, 1.06 M) in 60 mL dry ether at −78 °C under nitrogen atmosphere was added dropwise 4-methoxy acetophenone **24a** (3.0 g, 20 mmol) in 10 mL dry ether. The mixture was stirred at −78 °C for 45 min before diethyl oxalate (5.4 mL, 40 mmol) was added dropwise. After warmed to room temperature and stirred for another 3 h, yellow precipitation appeared. The mixture was allowed to settle overnight. The yellow solid was filtered and washed with 60 mL hot ether to afford a pale yellow powder which was suspended in water and acidified to pH 2–3 with 1 M hydrochloric acid in ice bath. Subsequent filtration afforded **25a** (1.32 g, 70% yield) as a yellow solid. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 15.47 (br s, 1H), 7.98 (d, 2H, $J$ = 8.0 Hz), 7.02 (s, 1H), 6.97 (d, 2H, $J$ = 8.0 Hz), 4.39 (q, 2H), 3.89 (s, 3H) and 1.41 (t, 3H) ppm. Mp: 246–248 °C.

#### 4.3.4. General procedure for the preparation of 25b–*d*

To the solution of acetophenones **24b–d** (1.0 g) in 10 mL dry toluene in ice bath was added NaH (1.5 equiv) under nitrogen atmosphere. The mixture was allowed to warm to room temperature and stir for another 1.5 h. After cooled in ice-water bath, diethyl oxalate (2.0 equiv) dissolved in 7 mL dry toluene was added dropwise. The mixture was heated at 50 °C for 15 min, poured into 100 mL ice water, and acidified to pH 5 with 1 M HCl. The aqueous layer was separated and extracted with ethyl acetate (30 mL × 2). The organic layers were combined, washed with water and brine, dried over anhydrous sodium sulfate, and evaporated. Further appropriate purification afforded the desired compounds **25b–d**.

**4.3.4.1. (*Z*)-Ethyl 4-(4-chlorophenyl)-2-hydroxy-4-oxo-2-butenoate (25b).** Compound **25b** was prepared from 4-chloroacetophenone **24b** (1.0 g, 6.47 mmol) and diethyl oxalate (1.890 g, 12.94 mmol) in the presence of NaH (388 mg, 9.71 mmol, 60% in mineral oil). The residue was washed with dichloromethane to give compound **25b** (0.728 g, 44.0% yield) as a yellowish solid. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 7.88 (d, 2H, $J$ = 7.2 Hz), 7.49 (d, 2H, $J$ = 7.2 Hz), 6.51 (br s, 1H), 4.16 (q, 2H) and 1.25 (t, 3H) ppm. Mp: 214–215 °C.

**4.3.4.2. (*Z*)-Ethyl 4-(2,4-difluorophenyl)-2-hydroxy-4-oxo-2-butenoate (25c).** Compound **25c** was prepared from 2,4-difluoroacetophenone **24c** (1.0 g, 6.41 mmol) and diethyl oxalate (1.873 g, 12.82 mmol) in the presence of NaH (384 mg, 9.61 mmol, 60% in mineral oil). The residue was washed by hexane to give compound **25c** (287 mg, 11.2% yield) as a yellow solid. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 15.05 (br s, 1H), 8.01 (m, 1H), 7.08 (d, 1H), 7.02 (m, 1H), 6.92 (m, 1H), 4.39 (q, 2H) and 1.40 (t, 3H) ppm.

**4.3.4.3. (*Z*)-Ethyl 2-hydroxy-4-(naphthalen-1-yl)-4-oxo-2-butenoate (25d).** Compound **25d** was prepared from 1-(naphthalen-1-yl) ethanone **24d** (1.0 g, 5.88 mmol) and diethyl oxalate (1.718 g, 11.76 mmol) in the presence of NaH (353 mg, 8.82 mmol, 60% in mineral oil). The residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate (5/1, v/v) as eluent to give compound **25d** (1.497 g, 92.4 % yield) as a yellow solid. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 15.15 (br s, 1H), 7.59 (d, 1H, $J$ = 8.0 Hz), 8.04 (d, 1H, $J$ = 8.0 Hz), 7.91 (m, 2H), 7.52–7.65 (m, 3H), 7.00 (s, 1H), 4.40 (q, 2H, $J$ = 7.2 Hz) and 1.41 (t, 3H, $J$ = 7.2 Hz) ppm. Mp: 59–60 °C.

#### 4.3.5. General procedure for the synthesis of compounds 26–31 and 34–45

The appropriate aldehyde (1.0–5.0 equiv) and amine (1.0 equiv) in ethanol or toluene was stirred at room temperature for 1 h before the appropriate diketo ester (0.7–2.0 equiv) was added. The mixture was heated to reflux for 24 h to provide 2-pyrrolinones **26–31** and **34–45**. For the synthesis of compounds **26, 28, 38** and **45**, DIPEA (0.1–0.2 equiv) was added as a catalyst. Purification:

Method A, the reaction mixture was filtered, washed, and recrystallized from appropriate solvent to provide the product; Method B, the solvent was removed by rotary evaporation and the product was purified by column chromatography on silica gel; Method C, the solvent was removed by rotary evaporation and the residue was purified by preparative HPLC.

**4.3.5.1. *tert*-Butyl 3-(2-(4-ethylphenyl)-4-hydroxy-3-(4-methoxybenzoyl)-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)propylcarbamate (26).** Compound **26** was prepared from 4-ethylbenzaldehyde (71 mg, 0.53 mmol), *tert*-butyl 3-aminopropylcarbamate (20 mg, 0.11 mmol) and **25a** (27 mg, 0.11 mmol) in the presence of DIPEA (1.9 mg, 0.014 mmol) in 5 mL ethanol. Purification with Method A using ethanol as recrystallization solvent gave compound **26** (28 mg, 49.2% yield) as a white solid. $^1$H NMR: (300 MHz, DMSO-$d_6$): 7.71 (d, 2H, $J$ = 8.7 Hz), 7.20 (d, 2H, $J$ = 8.1 Hz), 7.14 (d, 2H, $J$ = 7.8 Hz), 6.96 (d, 2H, $J$ = 9.0 Hz), 6.71 (t, 1H, $J$ = 4.2 Hz), 5.44 (s, 1H), 3.81 (s, 3H), 3.53 (m, 1H), 2.85 (m, 2H), 2.63 (m, 1H), 2.55 (q, 2H, $J$ = 7.5 Hz), 1.56 (m, 2H), 1.36 (s, 9H) and 1.12 (t, 3H, $J$ = 7.5 Hz) ppm. $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 187.50, 165.18, 162.91, 155.46, 149.64, 143.67, 133.20, 131.26, 131.16, 130.48, 127.97, 127.53, 120.15, 113.47, 113.41, 77.50, 60.67, 55.49, 38.08, 37.39, 28.19, 27.95, 27.69,15.13 ppm. Mp: 224–226 °C. HRMS (ESI): $[M+H]^+$ $C_{28}H_{34}N_2O_6$ calcd 495.2495, found 495.2508.

**4.3.5.2. *tert*-Butyl 4-(2-(4-ethylphenyl)-4-hydroxy-3-(4-methoxybenzoyl)-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)benzyl carbamate (27).** Compound **27** was prepared from 4-ethyl benzaldhyde (67 mg, 0.50 mmol), *tert*-butyl 4-aminobenzylcarbamate **23b** (111 mg, 0.50 mmol) and **25a** (125 mg, 0.50 mmol) in 5 mL ethanol. Purification with Method A using ethyl acetate/hexane (1/2, v/v) as recrystallization solvent gave compound **27** (13.0 mg, 4.8% yield) as a white solid. $^1$H NMR (400 MHz, MeOD): $\delta$ 7.78 (d, 2H, $J$ = 8.4 Hz), 7.52 (d, 2H, $J$ = 8.4 Hz), 7.22 (d, 2H, $J$ = 8.0 Hz), 7.20 (d, 2H, $J$ = 7.6 Hz), 7.02 (d, 2H, $J$ = 7.6 Hz), 6.96 (d, 2H, $J$ = 8.4 Hz), 6.19 (s, 1H), 4.15 (s, 2H), 3.86 (s, 3H), 2.49 (q, 2H, $J$ = 8.0 Hz), 1.44 (s, 9H) and 1.10 (t, 3H, $J$ = 7.6 Hz) ppm. $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 188.16, 165.04, 163.59, 156.23, 148.78, 143.64, 137.59, 135.42, 133.94, 131.84, 130.87, 128.19, 128.00, 127.66, 122.91, 121.32, 114.03, 78.26, 61.60, 55.98, 43.34, 28.70, 28.04, 15.34 ppm. Mp: 216–218 °C. HRMS (ESI): $[M+H]^+$ $C_{32}H_{34}N_2O_6$ calcd 543.2495, found 543.2508.

**4.3.5.3. *N*-3-(2-(4-Ethylphenyl)-4-hydroxy-3-(4-methoxybenzoyl)-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)propyl-3-methylbutyramide (28).** Compound **28** was prepared from 4-ethylbenzaldehyde (38 mg, 0.28 mmol), *N*-(3-aminopropyl)-3-methylbutyramide hydrochloride **23** (50 mg, 0.26 mmol) and **25a** (51.3 mg, 0.20 mmol) in the presence of DIPEA (1.9 mg, 0.014 mmol) in 10 mL ethanol. Purification with Method A using dichloromethane/hexane (1/3, v/v) as recrystallization solvent gave **28** (10.9 mg, 11.1% yield) as a white solid. $^1$H NMR: (400 MHz, MeOD): $\delta$ 7.77 (d, 2H, $J$ = 8.2 Hz), 7.18 (d, 2H, $J$ = 8.0 Hz), 7.14 (d, 2H, $J$ = 8.0 Hz), 6.93 (d, 2H, $J$ = 8.8 Hz), 5.55 (s, 1H), 3.84 (s, 3H), 3.65 (m, 1H), 3.05–3.20 (m, 2H), 2.86 (m, 1H), 2.57 (q, 2H, $J$ = 7.6 Hz), 2.01 (m, 3H), 1.66 (m, 2H), 1.16 (t, 3H, $J$ = 7.6 Hz) and 0.90 (d, 6H, $J$ = 5.2 Hz) ppm. $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 188.89, 171.31, 165.17, 162.77, 143.64, 132.06, 131.18, 131.03, 127.92, 127.50, 123.97, 114.20, 113.36, 60.69, 55.41, 54.83, 44.79, 38.22, 35.92, 27.80, 27.69, 25.40, 22.25, 15.11 ppm. Mp: 210–212 °C. HRMS (ESI): $[M+H]^+$ $C_{28}H_{34}N_2O_5$ calcd 479.2540, found 479.2554.

**4.3.5.4. 1-(Benzo[1,3]dioxol-5-yl-methyl)-5-(4-ethylphenyl)-3-hydroxy-4-(4-methoxybenzoyl)-1,5-dihydro-2*H*-pyrrol-2-one (29).** Compound **29** was prepared from 4-ethylbenzaldehyde (71 mg, 0.53 mmol), (benzo[1,3]dioxol-5-yl)methylamine (80 mg, 0.52 mmol) and **25a** (125 mg, 0.50 mmol) in 5 mL ethanol. Purification with Method A using dichloromethane/hexane (5/4, v/v) as recrystallization solvent gave compound **29** (55 mg, 21.0% yield) as a white solid. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.65 (d, 2H, $J$ = 8.0 Hz), 7.08 (d, 2H, $J$ = 7.6 Hz), 7.01 (d, 2H, $J$ = 8.0 Hz), 6.81 (d, 2H, $J$ = 8.0 Hz), 6.76 (d, 1H, $J$ = 8.0 Hz), 6.68 (s, 1H), 6.61 (d, 1H, $J$ = 8.0 Hz), 5.98 (s, 2H), 5.33 (s, 1H), 5.06 (d, 1H, $J$ = 14.8 Hz), 3.81 (s, 3H), 3.50 (d, 1H, $J$ = 14.8 Hz), 2.58 (q, 2H, $J$ = 8.0 Hz) and 1.18 (t, 3H, $J$ = 7.6 Hz) ppm. $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 187.16, 165.63, 162.77, 147.40, 146.49, 143.64, 131.23, 130.51, 127.92, 127.65, 121.12, 113.33, 108.16, 108.11, 100.95, 60.37, 55.40, 43.58, 27.70, 15.15 ppm. Mp: 263–265 °C. HRMS (ESI): $[M+H]^+$ $C_{28}H_{25}NO_6$ calcd 472.1755, found 472.1783.

**4.3.5.5. 4-(2-(4-Ethylphenyl)-4-hydroxy-3-(4-methoxybenzoyl)-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)methyl benzoic acid (30).** Compound **30** was prepared from 4-ethylbenzaldehyde (134 mg, 1.0 mmol), 4-aminomethylbenzoic acid (151 mg, 1.0 mmol) and **25a** (250 mg, 1.0 mmol) in 14 mL toluene. The resulting precipitate was filtered and washed with ethyl acetate (2 mL) and $H_2O$ (30 mL) to give **30** (170 mg, 34% yield) as a white solid. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 12.90 (br s, 1H), 11.75 (br s, 1H), 7.86 (d, 2H, $J$ = 8.0 Hz), 7.74 (d, 2H, $J$ = 8.4 Hz), 7.19 (d, 2H, $J$ = 7.6 Hz), 7.12 (d, 2H, $J$ = 8.0 Hz), 7.09 (d, 2H, $J$ = 8.0 Hz), 6.96 (d, 2H, $J$ = 8.4 Hz), 5.24 (s, 1H), 4.86 (d, 1H, $J$ = 15.6 Hz), 3.87 (d, 1H, $J$ = 15.6 Hz), 3.81 (s, 3H), 2.53 (q, 2H, $J$ = 7.6 Hz), 1.11 (t, 3H, $J$ = 7.6 Hz) ppm. $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 187.06, 166.97, 162.72, 157.15, 150.71, 143.69, 141.72, 133.20, 131.20, 130.68, 129.73, 129.52, 129.44, 127.90, 127.69, 127.57, 119.50, 113.31, 60.89, 55.39, 43.76, 27.70, 15.18 ppm. Mp: 260–263 °C. HRMS (ESI): $[M+H]^+$ $C_{28}H_{25}NO_6$ calcd 472.1755, found 472.1783.

**4.3.5.6. 5-(4-Ethylphenyl)-3-hydroxy-1-(3-imidazol-1-yl-propyl)-4-(4-methoxybenzoyl)-1,5-dihydro-2*H*-pyrrol-2-one (31).** Compound **31** was prepared from 4-ethylbenzaldehyde (67 mg, 0.50 mmol), 3-imidazol-1-yl propylamine (62 mg, 0.50 mmol) and **25a** (125 mg, 1.0 mmol) in 5 mL ethanol. Purification with Method C gave **31** (9 mg, 3.46% yield) as a brown solid. $^1$H NMR: (300 MHz, MeOD): $\delta$ 8.98 (br s, 1H), 7.80 (d, 2H, $J$ = 8.4 Hz), 7.66 (s, 1H), 7.56 (s, 1H), 7.22 (d, 2H, $J$ = 7.5 Hz), 7.16 (d, 2H, $J$ = 7.2 Hz), 6.94 (d, 2H, $J$ = 7.5 Hz), 5.57 (s, 1H), 4.21 (s, 2H), 3.84 (s, 3H), 3.63 (m, 1H), 2.95 (m, 1H), 2.58 (q, 2H, $J$ = 7.5 Hz), 2.04 (m, 2H) and 1.15 (t, 3H, $J$ = 7.5 Hz) ppm. $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 187.50, 165.48, 162.93, 154.48, 149.47, 143.80, 135.41, 133.03, 131.20, 130.41, 128.00, 127.58, 121.73, 120.30, 120.04, 113.42, 60.42, 55.42, 45.95, 36.83, 27.94, 27.65, 15.11 ppm. Mp: 115–117 °C. HRMS (ESI): $[M+H]^+$ $C_{26}H_{28}N_2O_4$ calcd 446.2074, found 446.2092.

**4.3.5.7. 1-(3-Aminopropyl)-5-(4-ethylphenyl)-3-hydroxy-4-(4-methoxy-benzoyl)-1,5-dihydro-2*H*-pyrrol-2-one (32).** Compound **26** (20 mg, 0.04 mmol) was treated with dry hydrochloride dichloromethane solution. After the reaction was completed, the solid was filtered and washed by dichloromethane to give **32** (11 mg, 63.2% yield). $^1$H NMR: (400 MHz, DMSO-$d_6$): $\delta$ 7.88 (br s, 3H), 7.72 (d, 2H, $J$ = 8.4 Hz), 7.22 (d, 2H, $J$ = 7.6 Hz), 7.16 (d, 2H, $J$ = 8.0 Hz), 6.96 (d, 2H, $J$ = 8.8 Hz), 5.47 (s, 1H), 3.81 (s, 3H), 3.61 (m, 1H), 2.70 (m, 3H), 2.52 (q, 2H, $J$ = 7.6 Hz), 1.74 (m, 2H) and 1.12 (t, 3H, $J$ = 7.6 Hz) ppm. $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 188.06, 165.98, 162.82, 149.50, 143.65, 136.79, 134.10, 131.18, 127.98, 127.52, 119.93, 115.27, 113.35, 60.53, 55.40, 37.44, 36.56, 27.66, 25.68, 15.07 ppm. Mp: 242–243 °C. HRMS (ESI): $[M+H]^+$ $C_{23}H_{27}N_2O_4$ calcd 395.1965, found 395.1980.

**4.3.5.8. 1-(4-Aminomethylphenyl)-5-(4-ethylphenyl)-3-hydroxy-4-(4-methoxybenzoyl)-1,5-dihydro-2*H*-pyrrol-2-one (33).** Treatment of compound **27** (40 mg, 0.074 mmol) with dry hydrochloride dichloromethane solution as described above for the preparation of **32** provided **33** (30 mg, 84.0% yield). $^1$H NMR: (400 MHz, DMSO-$d_6$): $\delta$ 11.53 (br s, 1H), 8.29 (br s, 3H), 7.73 (d, 2H, $J$ = 8.4 Hz), 7.68 (d, 2H, $J$ = 8.0 Hz), 7.42 (d, 2H, $J$ = 8.0 Hz), 7.28 (d, 2H, $J$ = 8.0 Hz), 7.02 (d, 2H, $J$ = 7.6 Hz), 6.99 (d, 2H, $J$ = 8.4 Hz), 6.29 (s, 1H), 3.93 (s, 2H), 3.82 (s, 3H), 2.43 (q, 2H, $J$ = 7.6 Hz) and 1.04 (t, 3H, $J$ = 7.6 Hz) ppm. $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 187.07, 165.50, 162.73, 158.55, 142.90, 136.66, 134.20, 131.25, 130.77, 130.48, 130.22, 129.44, 129.31, 127.53, 122.10, 120.43, 113.34, 60.79, 55.46, 41.64, 27.51, 14.94 ppm. Mp: 248–250 °C. HRMS (ESI): $[M+H]^+$ $C_{27}H_{27}N_2O_4$ calcd 443.1971, found 443.1985.

**4.3.5.9. 3-Hydroxy-1-(2-(indol-3-yl)ethyl)-4-(4-methoxybenzoyl)-5-(4-trifluoromethylphenyl)-1,5-dihydro-2*H*-pyrrol-2-one (34).** Compound **34** was prepared from 4-trifluoromethylbenzaldehyde (50 mg, 0.50 mmol), tryptamine (80 mg, 0.50 mmol) and **25a** (125 mg, 0.50 mmol) in 4 mL ethanol. Purification with Method C gave **34** (12 mg, 4.6% yield) as a brown solid. $^1$H NMR (300 MHz, MeOD): $\delta$ 7.55 (d, 2H, $J$ = 8.7 Hz), 7.40–7.46 (m, 3H), 7.32 (d, 1H, $J$ = 7.8 Hz), 7.14 (dd, 1H, $J_1$ = $J_2$ = 7.5 Hz), 6.75–7.05 (m, 4H), 6.88 (d, 2H, $J$ = 8.4 Hz), 5.04 (s, 1H), 3.97 (m, 1H), 3.83 (s, 3H) and 2.92–3.11 (m, 3H) ppm. $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 187.14, 165.33, 162.78, 151.84, 147.77, 143.92, 141.47, 136.19, 131.07, 128.80, 128.71, 128.62, 126.84, 125.31, 122.75, 120.94, 118.12, 117.90, 113.32, 111.37, 110.73, 60.59, 55.37, 41.05, 23.60 ppm. Mp: 215–217 °C. HRMS (ESI): $[M+H]^+$ $C_{29}H_{23}F_3N_2O_4$ calcd 521.1688, found 521.1696.

**4.3.5.10. 3-Hydroxy-1-(2-(indol-3-yl)ethyl)-5-(4-isopropylphenyl)-4-(4-methoxybenzoyl)-1,5-dihydro-2*H*-pyrrol-2-one (35).** Compound **35** was prepared from 4-isopropylbenzaldehyde (74 mg, 0.50 mmol), tryptamine (80 mg, 0.50 mmol) and **25a** (125 mg, 0.50 mmol) in 8 mL toluene. The precipitate was filtered and washed by toluene (1 mL × 2) to give **35** (78 mg, 31.6% yield) as a white solid. $^1$HNMR (400 MHz, $CDCl_3$) : $\delta$ 8.06 (br s, 1H), 7.48 (d, 2H, $J$ = 8.8 Hz), 7.40 (d, 2H, $J$ = 8.0 Hz), 7.22 (dd, 1H, $J_1$ = $J_2$ = 8.0 Hz), 7.10 (dd, 1H, $J_1$ = $J_2$ = 7.2 Hz), 7.04 (s, 1H), 7.03 (d, 2H, $J$ = 7.6 Hz), 6.84 (d, 2H, $J$ = 8.0 Hz), 6.77 (d, 2H, $J$ = 8.8 Hz), 5.16 (s, 1H), 4.02 (m, 1H), 3.81 (s, 3H), 3.11 (m, 2H), 2.90 (m, 1H), 2.79 (m, 1H) and 1.15 (d, 6H, $J$ = 6.0 Hz) ppm. $^{13}$C NMR (100 MHz, DMSO-$d_6$ ): $\delta$ 187.47, 164.90, 162.86, 149.88, 148.31, 136.28, 133.28, 131.17, 130.48, 127.68, 126.88, 126.43, 122.67, 120.90, 119.98, 118.12, 117.96, 113.39, 111.33, 110.81, 60.96, 55.39, 40.93, 32.96, 23.63 ppm. Mp: 239–241 °C. HRMS (ESI): $[M+H]^+$ $C_{31}H_{30}N_2O_4$ calcd 495.2284, found 495.2306.

**4.3.5.11. 5-(4-*tert*-Butylphenyl)-3-hydroxy-1-(2-(indol-3-yl)ethyl)-4-(4-methoxybenzoyl)-1,5-dihydro-2*H*-pyrrol-2-one (36).** Compound **36** was prepared from 4-*tert*-butylbenzaldehyde (81 mg, 0.5 mmol), tryptamine (80 mg, 0.5 mmol) and **25a** (125 mg, 0.5 mmol) in 8 mL toluene. Purification with Method A using ethanol as recrystallization solvent gave **36** (36 mg, 14.2% yield) as a white solid. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.04 (br s, 1H), 7.48 (d, 2H, $J$ = 8.8 Hz), 7.40 (dd, 2H, $J_1$ = 8.4 Hz, $J_2$ = 2.0 Hz), 7.22 (dd, 1H, $J_1$ = $J_2$ = 8.0 Hz), 7.19 (d, 2H, $J$ = 8.4 Hz), 7.10 (dd, 1H, $J_1$ = $J_2$ = 7.6 Hz),, 7.04 (d, 1H, $J$ = 2.0 Hz), 6.85 (d, 2H, $J$ = 8.4 Hz), 6.77 (d, 2H, $J$ = 8.8 Hz), 5.17 (s, 1H), 4.02 (m, 1H), 3.81 (s, 3H), 3.11 (m, 2H), 2.90 (m, 1H) and 1.22 (s, 9H) ppm. $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 187.45, 164.96, 162.86, 150.61, 136.18, 133.00, 131.22, 130.51, 127.49, 126.88, 125.31, 122.71, 120.93, 119.86, 118.12, 117.98, 113.41, 111.35, 110.80, 60.88, 55.42, 40.98, 34.22, 31.02, 23.60 ppm. Mp: 264–266 °C. HRMS (ESI): $[M+H]^+$ $C_{32}H_{32}N_2O_4$ calcd 509.2435, found 509.2443.

**4.3.5.12. 5-(4-Butylphenyl)-3-hydroxy-1-(2-(indol-3-yl)ethyl)-4-(4-methoxybenzoyl)-1,5-dihydro-2*H*-pyrrol-2-one (37).** Compound **37** was prepared from 4-butylbenzaldehyde (280 mg, 1.5 mmol), tryptamine (240 mg, 1.5 mmol) and **25a** (375 mg, 1.5 mmol) in 15 mL ethanol. Purification with Method A and use of ether (20 mL×2) to wash the precipitate twice gave **37** (214 mg, 28.1% yield) as an off white solid. $^1$H NMR: (400 MHz, DMSO-$d_6$): $\delta$ 11.48 (br s, 1H), 10.79 (s, 1H), 7.69 (d, 2H, $J$ = 8.4 Hz), 7.33 (d, 1H, $J$ = 8.0 Hz), 7.27 (d, 1H, $J$ = 6.4 Hz), 7.04–7.12 (m, 6H), 6.90–6.97 (m, 3H), 5.35 (s, 1H), 3.81 (s, 4H), 2.96 (m, 2H), 2.72 (m, 1H), 2.51 (t, 2H, $J$ = 7.6 Hz), 1.50 (m, 2H), 1.25 (m, 2H) and 0.85 (t, 3H, $J$ = 7.2 Hz) ppm. $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 187.35, 165.19, 162.77, 142.33, 136.22, 133.44, 131.19, 130.65, 128.41, 127.65, 126.92, 122.71, 120.96, 119.67, 118.18, 118.05, 113.36, 111.39, 110.88, 60.98, 55.42, 40.91, 34.45, 32.92, 23.70, 21.76, 13.70 ppm. Mp 179–182 °C. HRMS (ESI): $[M+H]^+$ $C_{32}H_{32}N_2O_4$ calcd 509.2435, found 509.2444.

**4.3.5.13. 3-Hydroxy-1-(2-(indol-3-yl)ethyl)-5-(4-isobutylphenyl)-4-(4-methoxybenzoyl)-1,5-dihydro-2*H*-pyrrol-2-one (38).** Compound **38** was prepared from 4-isobutylbenzaldehyde (405 mg, 2.5 mmol), tryptamine (80 mg, 0.5 mmol) and **25a** (125 mg, 0.5 mmol) in the presence of DIPEA (6.5 mg, 0.05 mmol) in 8 mL toluene. Purification with Method A using ethyl acetate/hexane (1/3, v/v) as recrystallization solvent gave **38** (33 mg, 12.9% yield) as an off-white solid. $^1$H NMR (300 MHz, $CDCl_3$) : $\delta$ 8.09 (br s, 1H), 7.38–7.44 (m, 4H), 7.22 (dd, 1H, $J_1$ = $J_2$ = 7.5 Hz), 7.10 (dd, 1H, $J_1$ = $J_2$ = 7.5 Hz), 7.02 (s, 1H), 6.93 (d, 2H, $J$ = 7.8 Hz), 6.76 (m, 4H), 5.13 (s, 1H), 4.02 (m, 1H), 3.79 (s, 3H), 3.10 (m, 2H), 2.90 (m, 1H), 2.35 (d, 2H, $J$ = 6.9 Hz), 1.73 (m, 1H) 0.80 (d, 3H, $J$ = 6.0 Hz) and 0.78 (d, 3H, $J$ = 6.0 Hz) ppm. $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 187.33, 165.18, 162.72, 141.12, 136.21, 133.56, 131.15, 130.69, 129.07, 127.50, 126.90, 122.71, 120.95, 119.56, 118.17, 118.03, 113.34, 111.38, 110.88, 60.98, 55.41, 44.20, 40.93, 29.46, 23.75, 22.12 ppm. Mp: 191–193 °C. HRMS (ESI): $[M+H]^+$ $C_{32}H_{32}N_2O_4$ calcd 509.2440, found 509.2449.

**4.3.5.14. 5-Biphenyl-4-yl-3-hydroxy-1-(2-(indol-3-yl)ethyl)-4-(4-methoxybenzoyl)-1,5-dihydro-2*H*-pyrrol-2-one (39).** Compound **39** was prepared from 4-phenylbenzaldehyde (91 mg, 0.50 mmol), tryptamine (80 mg, 0.50 mmol) and **25a** (125 mg, 0.50 mmol) in 2.5 mL toluene. Purification with Method A using dichloromethane/MeOH/ether (1/1/8, v/v/v) as recrystallization solvent gave **39** (32.0 mg, 12.1% yield) as a yellowish solid. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 10.83 (s, 1H), 7.59–7.65 (m, 4H), 7.49 (d, 2H, $J$ = 7.2 Hz), 7.42 (dd, 2H, $J_1$ = $J_2$ = 7.2 Hz), 7.30–7.34 (m, 3H), 7.03–7.14 (m, 4H), 6.92 (1H, dd, $J_1$ = $J_2$ = 7.6 Hz), 6.78 (d, 2H, $J$ = 7.6 Hz), 5.24 (s, 1H), 3.80 (m, 1H), 3.75 (s, 3H), 2.97 (m, 1H), 2.83 (m, 1H) and 2.71 (m, 1H) ppm. $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 186.18, 166.77, 160.27, 142.35, 140.61, 139.98, 138.63, 136.21, 133.92, 130.18, 129.31, 128.80, 128.12, 127.14, 126.59, 126.46, 126.03, 123.66, 123.26, 122.62, 120.90, 118.17, 112.17, 111.35, 111.31, 60.56, 55.05, 40.80, 23.53 ppm. Mp: 258–260 °C. HRMS (ESI): $[M+H]^+$ $C_{34}H_{28}N_2O_4$ calcd 529.2127, found 529.2140.

**4.3.5.15. 3-Hydroxy-1-(2-(indol-3-yl)ethyl)-4-(4-methoxybenzoyl)-5-naphthalen-2-yl-1,5-dihydro-2*H*-pyrrol-2-one (40).** Compound **40** was prepared from 2-naphthaldehyde (156 mg, 0.5 mmol), tryptamine (40 mg, 0.25 mmol) and **25a** (62.5 mg, 0.25 mmol) in the presence of DIPEA (6.5 mg, 0.05 mmol) in 8 mL toluene. Purification with Method B using ethyl acetate/MeOH/AcOH (20/1/0.4, v/v/v) as eluent to gave **40** (15.4 mg,

11.9% yield) as an off-white solid. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.65 (br s, 1H), 10.84 (s, 1H), 7.81–7.86 (m, 3H), 7.74 (s, 1H), 7.70 (d, 2H, $J$ = 8.4 Hz), 7.50 (m, 2H), 7.33(d, 1H, $J$ = 8.4 Hz), 7.27 (d, 1H, $J$ = 8.4 Hz), 7.19 (d, 1H, $J$ = 8.0 Hz), 7.12 (s, 1H), 7.04 (dd, 1H, $J_1 = J_2$ = 7.6 Hz), 6.94 (d, 2H, $J$ = 8.8 Hz), 6.79 (dd, 1H, $J_1 = J_2$ = 7.6 Hz), 5.47 (s, 1H), 3.86 (m, 1H), 3.79 (s, 3H), 2.73–3.05 (m, 2H) and 2.76 (m, 1H) ppm. $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 187.45, 172.54, 162.77, 148.52, 142.31, 137.58, 136.20, 132.74, 132.68, 131.12, 129.91, 128.33, 128.14, 127.62, 127.45, 126.84, 126.26, 126.20, 123.98, 122.78, 120.92, 118.14, 118.05, 113.34, 111.37, 110.85, 61.38, 55.38, 40.90, 23.83 ppm. Mp: 233–234 °C. HRMS (ESI): $[M+H]^+$ $C_{32}H_{26}N_2O_4$ calcd 503.1971, found 503.1980.

**4.3.5.16. 5-(2-Bromo-4-fluorophenyl)-3-hydroxy-1-(2-(indol-3-yl)ethyl)-4-(4-methoxybenzoyl)-1,5-dihydro-2*H*-pyrrol-2-one (41).** Compound **41** was prepared from 2-bromo-4-fluorobenzaldehyde (102 mg, 0.50 mmol), tryptamine (80 mg, 0.50 mmol), and **20a** (125 mg, 0.50 mmol) in 8 mL ethanol. Purification with Method A using dichloromethane/MeOH (2/1, v/v) as recrystallization solvent gave **41** (58 mg, 21.1% yield) as an off white solid. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 10.79 (s, 1H), 7.84 (d, 2H, $J$ = 7.6 Hz), 7.46 (dd, 1H, $J_1$ = 8.4 Hz, $J_2$ = 2 Hz), 7.41 (d, 1H, $J$ = 8.0 Hz), 7.31 (d, 1H, $J$ = 8.0 Hz), 7.02–7.13 (m, 4H), 6.95 (dd, 1H, $J_1 = J_2$ = 7.2 Hz), 6.79 (d, 2H, $J$ = 8.8 Hz), 5.73 (s, 1H), 3.76 (s, 3H), 3.70 (m, 1H), 2.98 (m, 1H), 2.79 (m, 1H) and 2.65 (m, 1H) ppm. $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 170.08, 168.15, 161.74, 160.33, 159.27, 138.89, 137.56, 136.26, 133.98, 130.24, 129.11, 129.03, 127.10, 125.49, 125.39, 122.61, 120.96, 118.68, 118.44, 118.27, 118.10, 115.32, 115.11, 112.23, 111.44, 111.15, 105.22, 101.19, 58.58, 55.14, 41.36, 23.84 ppm. Mp: 317–319 °C. HRMS (ESI): $[M+H]^+$ $C_{28}H_{22}BrFN_2O_4$ calcd 549.0825, found 549.0844.

**4.3.5.17. 3-Hydroxy-1-(2-(indol-3-yl)ethyl)-4-(4-methoxybenzoyl)-5-pentyl-1,5-dihydro-2*H*-pyrrol-2-one (42).** Compound **42** was prepared from hexanal (50 mg, 0.50 mmol), tryptamine (80 mg, 0.50 mmol) and **25a** (125 mg, 0.50 mmol) in 4 mL toluene. Purification with Method C gave **42** (10 mg, 2.23% yield) as a brown solid. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.13 (br s, 1H), 7.63 (m, 3H), 7.39 (d, 1H, $J$ = 6.4 Hz), 7.22 (dd, 1H, $J_1 = J_2$ = 7.2 Hz), 7.14 (dd, 1H, $J_1 = J_2$ = 7.2 Hz), 7.08 (br s, 1H), 6.91 (d, 2H, $J$ = 8.4 Hz), 4.53 (m, 1H), 4.26 (m, 1H), 3.87 (s, 3H), 3.37 (m, 1H), 3.05–3.21 (m, 2H), 1.64 (m, 2H), 0.98–1.12 (m, 4H), 0.85 (m, 2H) and 0.74 (t, 3H, $J$ = 6.8 Hz) ppm. $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 188.29, 165.03, 162.85, 150.14, 136.22, 131.22, 131.07, 130.57, 127.03, 122.92, 121.11, 118.35, 118.16, 113.61, 111.51, 110.98, 56.52, 55.58, 40.56, 30.96, 27.45, 23.74, 21.79, 21.18, 13.73 ppm. Mp: 80–82 °C. HRMS (ESI): $[M+H]^+$ $C_{27}H_{30}N_2O_4$ calcd 447.2284, found 447.2297.

**4.3.5.18. 4-(4-Chlorobenzoyl)-3-hydroxy-1-(2-(indol-3-yl)ethyl)-5-(4-isobutylphenyl)-1,5-dihydro-2*H*-pyrrol-2-one (43).** Compound **43** was prepared from 4-isobutylbenzaldehyde (97 mg, 0.6 mmol), tryptamine (80 mg, 0.50 mmol) and **25b** (128 mg, 0.50 mmol) in 15 mL ethanol. Purification with Method B using dichloromethane/MeOH (10/1, v/v) as eluent gave **43** (14 mg, 5.4% yield) as a brown solid. $^1$HNMR (400 MHz, MeOD): $\delta$ 7.33–7.43 (m, 6H), 6.98–7.12 (m, 5H), 6.82 (m, 1H), 4.98 (s, 1H), 3.90 (m, 1H), 3.08 (m, 2H), 2.87 (m, 1H), 2.38 (d, 2H, $J$ = 6.0 Hz), 1.76 (m, 1H) and 0.82 (t, 6H, $J$ = 5.2 Hz) ppm. Mp: 215–217 °C. $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 175.81, 169.60, 159.69, 140.31, 139.24, 136.24, 134.44, 129.51, 128.53, 127.40, 127.33, 126.91, 123.05, 122.77, 120.97, 118.24, 117.97, 115.59, 111.46, 110.89, 61.47, 44.10, 41.29, 31.48, 29.47, 23.57, 21.99 ppm. HRMS (ESI): $[M+H]^+$ $C_{31}H_{29}N_2O_3$ calcd 513.1945, found 513.1962.

**4.3.5.19. 4-(2,4-Diflorophenyl)-3-hydroxy-1-(2-(indol-3-yl)ethyl)-5-(4-isobutylphenyl)-1,5-dihydro-2*H*-pyrrol-2-one (44).** Compound **44** was prepared from 4-isobutylbenzaldehyde (81 mg, 0.50 mmol), tryptamine (80 mg, 0.50 mmol) and **25c** (114 mg, 0.50 mmol) in 15 mL ethanol. Recrystallization gave **44** (57 mg, 22.1 % yield) as an off white solid. $^1$H NMR: (400 MHz, $CDCl_3$): 8.15 (dd, 1H, $J_1$ = 8.8 Hz, $J_2$ = 6.0 Hz), 8.06 (br s, 1H), 7.35–7.39 (m, 3H), 7.13–7.21 (m, 2H), 7.01–7.10 (m, 4H), 6.96 (d, 2H, $J$ = 8 Hz), 5.17 (s, 1H), 4.19 (m, 1H), 2.95–3.28 (m, 3H), 2.43 (d, 2H, $J$ = 7.2 Hz), 1.83 (m, 1H), 0.88 (d, 6H, $J$ = 6.4 Hz) ppm. $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 172.25, 166.93, 164.42, 161.17, 157.72, 157.58, 155.70, 142.32, 136.73, 131.47, 129.79, 128.57, 128.47, 128.31, 127.83, 127.34, 123.41, 122.56, 121.50, 118.92, 118.73, 118.55, 118.46, 115.27, 115.04, 111.93, 111.16, 106.79, 106.53, 59.88, 44.74, 41.58, 30.00, 24.24, 22.61 ppm. Mp: 172–174 °C. HRMS (ESI): $[M+H]^+$ $C_{31}H_{28}F_2N_2O_3$ calcd 515.2146, found 515.2142.

**4.3.5.20. 5-(4-Ethylphenyl)-3-hydroxy-1-(2-(indol-3-yl)ethyl)-4-(naphthalene-1-carbonyl)-1,5-dihydro-2*H*-pyrrol-2-one (45).** Compound **45** was prepared from 4-ethylbenzaldehyde (340 mg, 2.53 mmol), tryptamine (80 mg, 0.50 mmol) and **25d** (135 mg, 0.5 mmol) in the presence of DIPEA (6.5 mg, 0.05 mmol) in 10 mL toluene. Purification with Method A using dichloromethane/hexane (1/2, v/v) as recrystallization solvent gave **45** (101 mg, 50.5% yield) as an off white solid. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 10.85 (s, 1H), 7.93–8.00 (m, 2H), 7.83 (d, 1H, $J$ = 7.8 Hz), 7.43–7.49 (m, 4H), 7.35 (d, 1H, $J$ = 8.1 Hz), 7.29 (d, 1H, $J$ = 7.8 Hz), 7.05–7.16 (m, 6H), 6.93 (dd, 1H, $J_1 = J_2$ = 7.2 Hz), 5.34 (s, 1H), 3.80 (m, 1H), 2.96 (m, 2H), 2.75 (m, 1H), 2.56 (q, 2H, $J$ = 7.2 Hz) and 1.15 (t, 3H, $J$ = 7.2 Hz) ppm. $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 186.81, 165.46, 162.75, 147.71, 144.08, 137.85, 136.75, 134.28, 133.55, 130.88, 129.96, 128.68, 128.33, 128.14, 127.48, 127.26, 126.93, 126.47, 125.35, 125.11, 123.26, 121.48, 118.72, 118.54, 111.92, 111.44, 61.17, 41.52, 28.27, 24.07, 15.80 ppm. Mp: 203–204 °C. HRMS (ESI): $[M+H]^+$ $C_{33}H_{28}N_2O_3$ calcd 501.2178, found 501.2188.

### 4.4. Aminoacylation assay

Experiments were performed in a final 70 μL reaction mixtures containing 50 mM HEPES-KOH (pH 7.8), 5 mM $MgCl_2$, 45 mM KCl, 1 mM DTT, 0.2 mg/mL BSA, 0.4 mg/mL brewer's yeast tRNA (Roche), 0.5 nM *Tb*LeuRS, 4 mM ATP, and 0.003 mCi/mL [$^{14}$C]-Leucine. The test compounds were dissolved in 7 μL DMSO (Genview) and added to the above mixture. The final compound concentration gradient was 1 mM, 320 μM, 100 μM, 32 μM, 10 μM, 3.2 μM, 1 μM, 0.32 μM, 0.1 μM. Reactions were initiated by the addition of ATP. At specific time intervals, three 20 μL aliquots were spotted on 3 mm filter paper (Whatman), washed three times in 5% trichloroacetic acid and three times in ethanol. Filter paper was dried under a heat lamp and the precipitated [$^{14}$C]-Leucine $tRNA^{Leu}$ were quantified using a Beckman Coulter LS 6500 liquid scintillation counter. The inhibitory effect for each compound was determined by incubating *Tb*LeuRS with the compound, tRNA and [$^{14}$C]-Leucine for 20 min at 37 °C prior to the addition of ATP to start the aminoacylation reaction. Triplicates were averaged to generate an $IC_{50}$ value using GraphPad.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.12.035.

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