European Journal of Medicinal Chemistry xxx (xxxx) xxx

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Design and synthesis of α-phenoxy-*N*-sulfonylphenyl acetamides as *Trypanosoma brucei* Leucyl-tRNA synthetase inhibitors

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ARTICLE INFO

Article history:
Received 25 June 2019
Received in revised form
21 October 2019
Accepted 26 October 2019
Available online xxx

Keywords:
Human African trypanosomiasis (HAT)
Anti-trypanosomal agent
Leucyl-tRNA synthetase (LeuRS)
α-phenoxy-*N*-Sulfonylphenyl acetamides
Structure-activity relationship

ABSTRACT

Human African trypanosomiasis (HAT), caused by the parasitic protozoa *Trypanosoma brucei*, is one of the fatal diseases in tropical areas and current medicines are insufficient. Thus, development of new drugs for HAT is urgently needed. Leucyl-tRNA synthetase (LeuRS), a recently clinically validated antimicrobial target, is an attractive target for development of antitrypanosomal drugs. In this work, we report a series of α-phenoxy-*N*-sulfonylphenyl acetamides as *T. brucei* LeuRS inhibitors. The most potent compound **28g** showed an IC_{50} of 0.70 μM which was 250-fold more potent than the starting hit compound **1**. The structure-activity relationship was also discussed. These acetamides provided a new scaffold and lead compounds for the further development of clinically useful antitrypanosomal agents.

1. Introduction

Human African trypanosomiasis (HAT), also known as sleeping sickness, is a parasitic disease caused by the protozoa *Trypanosoma brucei* [1]. It is transmitted by the tsetse fly through biting and threatens 61 million people in 36 countries in Africa. It is a fatal disease if not treated effectively in a timely manner. Up to now, there are only four drugs available in the clinics for the treatment of HAT: pentamidine and suramin for the early stage; melarsoprol and eflornithine for the late stage of the disease [2]. Although these drugs have been used for decades, there are still limitations such as serious toxic side effects, low efficacy, high cost, and difficulty of administration. Fexinidazole, a nitroimidazole approved in 2019 for the treatment of trypanosomiasis, will add to the existing arsenal [3]. However, the discovery of antitrypanosomal drugs has been challenging with eflornithine as the only new drug approved during the past 28 year until the approval of fexinidazole this year. Together with the emergence of drug resistance, thus new antitrypanosomal drugs are urgently needed [4,5].

Aminoacyl-tRNA synthetases (aaRSs) catalyze the attachment of amino acids to the 3′-hydroxyl of the terminal adenosine of the corresponding tRNA to produce the aminoacyl-tRNA, which happens in the synthetic active site of aaRSs [6]. Since the accuracy of the incorporation of the correct amino acid is essential in ensuring the fidelity of the genetic code, many aaRS enzymes possess a proofreading mechanism which happens in the editing active site where the incorrect amino acids will be hydrolyzed. Due to its crucial role in protein synthesis, aaRSs of pathogens are promising targets for the development of anti-infective drugs [7–11]. In recent years, leucyl-tRNA synthetase (LeuRS) has been paid much attention as a clinically validated antimicrobial target. Tavaborole, a LeuRS inhibitor, was approved in 2014 as a topical treatment of fungal infection of the toenails, and good selectivity between fungal and human LeuRS was achieved in this case [12]. A number of compounds have been reported as LeuRS inhibitors (Fig. 1). Boron-containing compound **a** (GSK2251052) inhibits bacterial (*E. coli*) LeuRS by targeting its editing site and has been evaluated clinically [13–15], however, the development of this compound was encumbered due to emergence of resistance. *N*-leucinylbenzene-sulfonamides such as compound **b**, mimicking the endogenous substrate, are reported as *E. coli* LeuRS inhibitors [10]. We have also previously reported benzoxaborole-based *T. brucei* LeuRS (*Tb*LeuRS) inhibitors such as compound **c** which target the editing site [16].

We have also been making continuous efforts searching for inhibitors that target the synthetic site of *Tb*LeuRS. In our previous

Abbreviations: HAT, human African trypanosomiasis; *T. brucei*, *Trypanosoma brucei*; LeuRS, leucyl-tRNA synthetase; *Tb*LeuRS, *T. brucei* LeuRS; aaRS, aminoacyl-tRNA synthetases.

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https://doi.org/10.1016/j.ejmech.2019.111827
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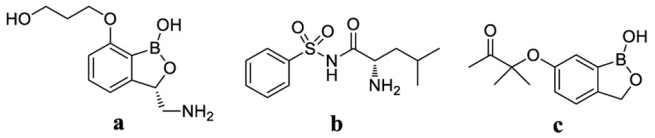

Fig. 1. Chemical structures of known LeuRS inhibitors.

screening of a collection of compounds from our laboratory, a *N*-(4-sulfonylphenyl)thiourea hit compound **1** ($IC_{50} = 174\ \mu M$) was identified as a new class of *Tb*LeuRS inhibitor which target the synthetic site (Fig. 2) [17]. Considering that the solubility of thiourea group is known to be relatively low and its toxicity also present an issue, which would limit further development of the scaffold, we took on a task to change the thiourea group to amide, and developed a series of α-phenoxy-*N*-sulfonylphenyl acetamides as *Tb*LeuRS inhibitors. The initially obtained acetamide **5** showed comparable activity to thiourea **1**. The terminal sulfonamide group in compound **5**, reminiscent of the leucyl area in the substrate mimics Leu-AMS (Fig. 3), were modified to give a ~5-fold increase of inhibitory activity (compound **19**, $IC_{50} = 38.4\ \mu M$). The observation that the 1,3-substitution pattern on the central phenyl ring would better resembles the geometry of Leu-AMS led to significantly improved potency as represented by compound **28g** ($IC_{50} = 0.70\ \mu M$). Thus, we successfully replaced thiourea with amide group and obtained good *Tb*LeuRS inhibitors with ~250-fold improvement of inhibitory activity from the initial hit compound.

2. Chemistry

Synthesis of α-phenoxy-*N*-(4-sulfonylphenyl)acetamides was shown in Scheme 1. First, ester **3** was prepared from 4-bromo-2-chlorophenol and ethyl 2-bromoacetate in the presence of potassium carbonate, which was subsequently hydrolyzed to give acid **4**. After conversion of acid **4** to its acyl chloride, it was coupled with 4-aminobenzenesulfonamide to yield compound **5**. It was further reacted with *N*-protected amino acids in the presence of EDCI and DMAP to give acetamides **6**–**8**, **10**, **13**, **15**, and **17**–**18**. Removal of the Boc group using HCl gas gave acetamides **9**, **14**, **16**, and **19**. Hydrolysis of the benzyl ester **10** under basic condition followed by acidification with HCl gas in dichloromethane gave acid **11**, while acidification with HCl gas in ethyl acetate gave ethyl ester **12**.

Synthesis of α-phenoxy-*N*-(3-sulfonylphenyl)acetamides **28a**-**g** was shown in Scheme 2. Esters **21a**-**g** were prepared from various phenols and ethyl bromoacetate in the presence of potassium carbonate, which was subsequently hydrolyzed to give acids **22a**-**g**. Cbz protection of aniline **23** gave compound **24** which was reacted with *N*-Boc-L-leucine in the presence of EDCI and DMAP as coupling reagent to give compound **25**. Compound **26** was obtained after the Cbz group was removed by 10% Pd/C and H_2, and was subsequently coupled with the acyl chlorides of **22a**-**g** to give compounds **27a**-**g**. Removal of the Boc group by HCl gas eventually gave compounds **28a**-**g**.

3. Results and discussion

Starting from the *N*-(4-sulfonylphenyl)thiourea hit compound **1**, which was identified from our laboratory collection as a new class of *Tb*LeuRS inhibitor that targets the synthetic site (Fig. 2), we decided to replace the thiourea group with amide group that is more desirable for further development. A direct replacement of the thiourea by amide group resulted in acetamide **5** that showed comparable activity to thiourea **1** (Table 1).

Next, we modified compound **5** by introducing various acyl groups onto the terminal amino group (Table 1). First, a heteroaryl compound **6** failed to improve potency, so we decided to make effort on amino acid-derived substituents. The phenylalanyl, glutamyl, glutaminyl, isoleucyl, leucyl-substituted compounds **7**–**19** were synthesized and tested for their *Tb*LeuRS inhibitory activity. It is rather interesting to see that the leucyl compound **19** showed the best inhibitory activity ($IC_{50} = 38.4\ \mu M$), which is a 5-fold improvement as compared to the initial acetamide **5**. In the case of the phenylalanyl, glutamyl, and isoleucyl compounds **7**–**12** and **15**–**16**, the benzyl or Boc-protected compounds showed better inhibitory activity than the free amino compounds. Among them, compounds **7** and **10** showed improved activity as compared to compound **5**. However, in the case of glutaminyl and leucyl compounds **13**–**14** and **17**–**19**, the compounds with free amino group showed better activity. The leucyl compound **19** gave significant improvement of inhibitory activity and represented the best compound in this round of effort. Therefore, the leucyl group was retained in the following study.

The molecule Leu-AMS mimicked the endogenous substrate Leu-AMP but contained a stable sulfonamide linkage group in place of the phosphate group (Fig. 3a). Thus, Leu-AMS is often used as a substrate mimic in protein-ligand crystallization studies. When compound **19** was superimposed with Leu-AMS, although the leucyl end of the two molecules overlapped well, the other end of the molecule deviated significantly from each other (Fig. 3b). This is due to the fact that Leu-AMS has the two extending branches spaced by a single oxygen atom in the sugar ring, while compound **19** has a 1,4-substitution pattern in its central phenyl ring. So, it was suggested that a 1,3-substitution pattern should make the geometry of our compounds more closely resembles Leu-AMS. Indeed, this strategy significantly improved the inhibitory activity as exemplified by comparing 1,4-substituted compound **29** ($IC_{50} = 43.6\ \mu M$) and its 1,3-substituted analog **28b** ($IC_{50} = 5.69\ \mu M$). The superimposed structures also showed that compound **28b** better resembles the geometry of Leu-AMS (Fig. 3c). A series of 1,3-substitued compounds **28a**-**g** were synthesized and evaluated as shown in Table 2. The phenyl and nitrophenyl compounds **28a** and **28c** gave comparable potency. In the case of the biphenyl compounds **28f** and **28g**, the 3′-biphenyl compound **28g** gave significantly improved inhibitory activity with an IC_{50} value of 0.70 μM, which represented the most potent compound in this study. Thus, we successfully replaced thiourea with amide group and obtained good *Tb*LeuRS inhibitors with ~250-fold improvement of inhibitory activity from the initial hit compound. The

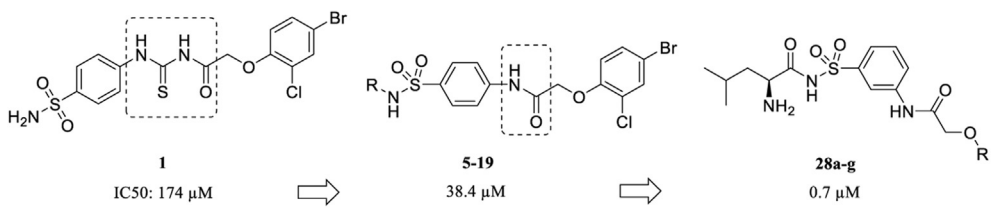

Fig. 2. Design of new LeuRS inhibitors with amide linkage group.

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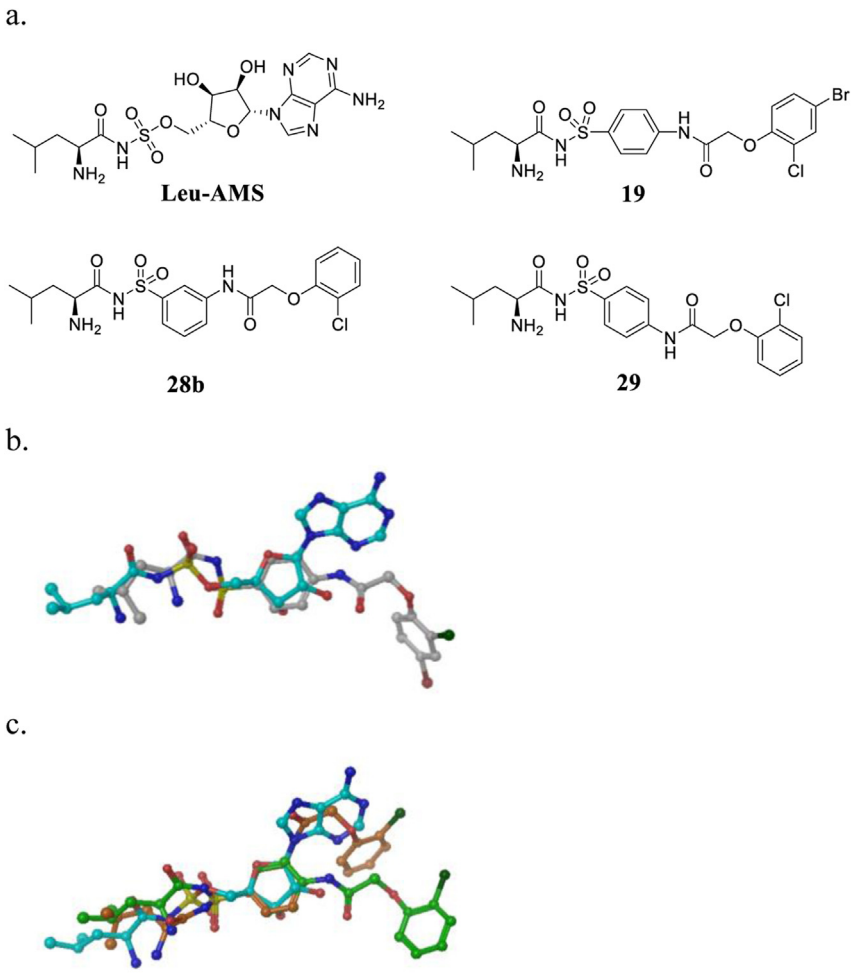

Fig. 3. a. Chemical structures of Leu-AMS and compounds **19**, **28b**, and **29**. b. Overlay of Leu-AMS (cyan) and compound **19** (grey). c. Overlay of Leu-AMS (cyan), compound **28b** (orange), and compound **29** (green). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

compounds were also tested on the *T. brucei* parasites, and among them compound **28f** showed inhibition of parasite growth with an IC_{50} of 33.33 ± 3.41 μM (SI Fig. 2). These compounds will be further optimized in terms of their physicochemical properties in order to achieve satisfactory antiparasitic activity in the future efforts.

4. Conclusions

As a neglected tropical disease, human African trypanosomiasis is in urgent need of new treatment, especially those in new chemical classes. LeuRS, as a clinically validated antimicrobial target, should serve as a promising antitrypanosomal target. Here, we reported the discovery of α-phenoxy-*N*-(3-sulfonylphenyl) acetamide as a new class of *Tb*LeuRS inhibitors with compound **28g** representing the most potent inhibitor in this study. This work successfully provided a new scaffold for further exploring new *Tb*LeuRS inhibitors, which may eventually become potential therapeutics for the treatment of African trypanosomiasis.

5. Material and methods

5.1. Chemistry

All solvents and reagents were purchased from commercial sources and used without further purification unless otherwise noted. Reactions were not optimized for maximum yields. Column chromatography was performed using Huanghai silica gel (45–75 μm). The preparative HPLC was conducted on Shanghai Unimicro EasySep-1010 liquid chromatography using a C_{18} column (30 mm × 250 mm), a 1 mL sample loop, a flow rate of 16 mL min^{-1}, and a gradient of 10% v/v MeOH in H_2O (0.1% v/v TFA) (t = 0 min) to 100% MeOH (t = 30.0 min). NMR spectra were recorded on Bruker Avance III 400 MHz. Chemical shifts are expressed in parts per million (ppm) relative to residual solvent as an internal reference ($CDCl_3$: 7.26; MeOD: 3.31; DMSO-d_6: 2.50). High resolution mass spectra were obtained on a Micromass GCT (electron ionization) or an Agilent 6530 Accurate Mass Q-TOF LC-MS (electrospray ionization). HPLC analysis was performed on an Agilent 1200 with a flow rate of 1 mL min^{-1} and two gradients: gradient A (10% v/v MeCN in H_2O (containing 0.1% v/v TFA) (t = 0.0 min) to 100% MeCN (t = 15.0 min)); gradient B (10% v/v MeCN in H_2O (t = 0.0 min) to 100% MeCN (t = 20.0 min)), stopping at 20 or 25 min using a DAD detector. An Agilent Eclipse XDB-C_{18} column (4.6 mm × 150 mm, 5 μm) was used. Purity was based on the integrated UV chromatogram (254 nm). The purity of all biological assay compounds was >95.0%.

5.1.1. Ethyl 2-(4-bromo-2-chlorophenoxy)acetate (**3**)

To a solution of 4-bromo-2-chlorophenol (**2**, 5.2 g, 25.0 mmol) and ethyl 2-bromoacetate (4.3 g, 25.7 mmol) in 50 mL DMF, anhydrous K_2CO_3 (3.5 g, 25.0 mmol) was added. After stirring overnight at 70 °C, the mixture was poured into 150 mL water and extracted

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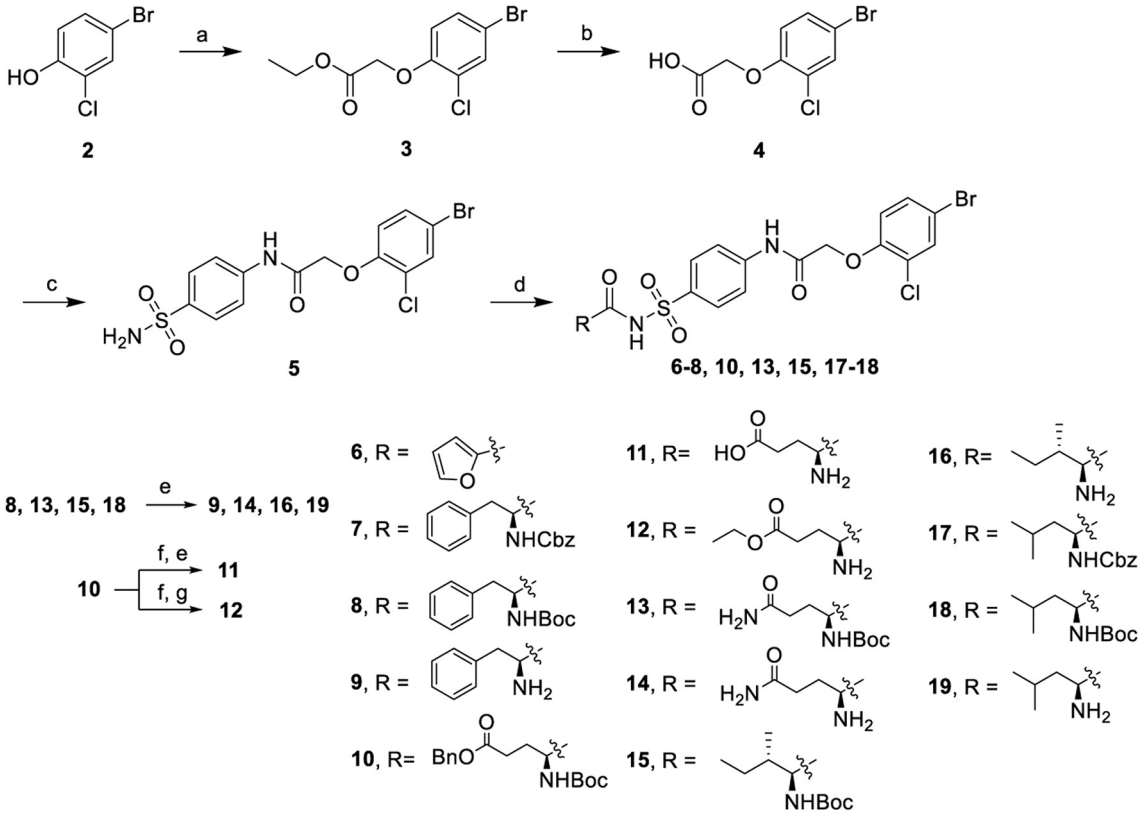

Scheme 1. Synthesis of N-(4-sulfamoylphenyl)thiourea derivatives. Condition and reagents: (a) ethyl 2-bromoacetate, K_2CO_3, DMF; (b) 1 M NaOH, dioxane; (c) $SOCl_2$, 4-aminobenzenesulfon amide; (d) RCOOH, EDCI, DMAP, DCM; (e) HCl gas, DCM; (f) 2.5 M NaOH, MeOH; (g) HCl gas, EtOAc.

with ethyl acetate (70 mL × 4). The organic layer was combined, washed with brine (100 mL × 3) and dried over anhydrous Na_2SO_4. The residue after rotary evaporation was purified by column chromatography to give **3** as an oil (6.18 g, 84.1% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (s, 1H), 7.30 (d, J = 8.4 Hz, 1H), 6.72 (d, J = 8.4 Hz, 1H), 4.68 (s, 2H), 4.26 (q, J = 7.2 Hz, 2H), 1.29 (t, J = 7.2 Hz, 3H).

5.1.2. 2-(4-Bromo-2-chlorophenoxy)acetic acid (*4*)

To a solution of **3** (5.0 g, 17.0 mmol) in 50 mL dioxane, 1 M NaOH (50 mL) was added. After stirring at r.t. overnight, the mixture was acidified with 1 M HCl to pH = 3. After extracted with ethyl acetate (50 mL × 4), the organic layer was washed with brine (50 mL), dried over anhydrous Na_2SO_4 and concentrated to give **4** as a white solid (4.69 g, 94.3% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.66 (s, 1H), 7.44 (d, J = 8.8 Hz, 1H), 6.97 (d, J = 8.8 Hz, 1H), 4.72 (s, 2H). Mp: 150–151 °C.

5.1.3. 2-(4′-Bromo-2′-chlorophenoxy)-N-(4′-sulfamoylphenyl) acetamide (*5*)

To a solution of compound **4** (300 mg, 1.13 mmol) in 6 mL $SOCl_2$ was added 2 drops DMF and heated to reflux. After 3 h, $SOCl_2$ was removed by atmospheric distillation. The residue, after dried *in vacuo* for 5 min, was dissolved in 15 mL dry acetone and added dropwise into sulfanilamide (182 mg, 1.07 mmol) in 225 mL acetone at 0 °C. The mixture was warmed to r.t. and continued stirring for 2 h. After the reaction completed, the mixture was filtered and the cake was washed with ether (10 mL × 2) to give compound **5** (405 mg, 85.4% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.54 (s, 1H), 7.83–7.73 (m, 4H), 7.71 (d, J = 2.4 Hz, 1H), 7.49 (dd, J = 8.9, 2.4 Hz, 1H), 7.27 (s, 2H), 7.08 (d, J = 8.9 Hz, 1H), 4.92 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.74, 153.51, 141.72, 139.29, 132.52, 131.41, 127.23, 123.28, 119.47, 116.33, 112.89, 68.12. Mp: 209–210 °C. HRMS: $[M+Na]^+$ calcd $[C_{14}H_{12}BrClN_2O_4S + Na]^+$ 440.9288, found 440.9306. HPLC: gradient A, 98.0% purity, 18.5 min.

5.1.4. General procedure for preparation of *6–8, 10, 13, 15, 17–18*

To a mixture of compound **5** (1.0 eq.) and corresponding carboxylic acid (1.2–2.0 eq.) in DCM EDCI (5.0 eq.) and DMAP (3.0 eq.) were added at 0 °C, then the mixture was warmed to r.t. and stirred overnight. The mixture was washed with 0.5 M HCl (30 mL × 3) and brine (30 mL), dried over anhydrous Na_2SO_4 and concentrated to give the residue which was purified by appropriate method to give the product.

5.1.5. N-((4-(2-(4′-Bromo-2′-chlorophenoxy)acetamido)phenyl) sulfonyl)furan-2-carboxamide (*6*)

Compound **6** (151 mg, 61.7% yield) was prepared from **5** (200 mg, 0.48 mmol) and furan-2-carboxylic acid (106 mg, 0.95 mmol) following the general procedure. The crude product was washed with DCM to give **6** as a white solid (151 mg, 61.7% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.43 (s, 1H), 10.64 (s, 1H), 7.95 (d, J = 9.1 Hz, 3H), 7.83 (d, J = 8.8 Hz, 2H), 7.71 (d, J = 2.4 Hz, 1H), 7.51 (d, J = 3.6 Hz, 1H), 7.48 (dd, J = 8.9, 2.4 Hz, 1H), 7.06 (d, J = 8.9 Hz, 1H), 6.68 (dd, J = 3.4, 1.4 Hz, 1H), 4.92 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.96, 153.47, 148.31, 145.37, 143.41, 134.09, 132.51, 131.41, 129.56, 123.24, 119.37, 118.34, 116.28, 112.84, 68.06, 55.38. Mp: 262–263 °C. HRMS: $[M+Na]^+$ calcd $[C_{19}H_{14}BrClN_2O_6S + Na]^+$ 534.9342, found 534.9352. HPLC: gradient A, 98.4% purity, 14.7 min.

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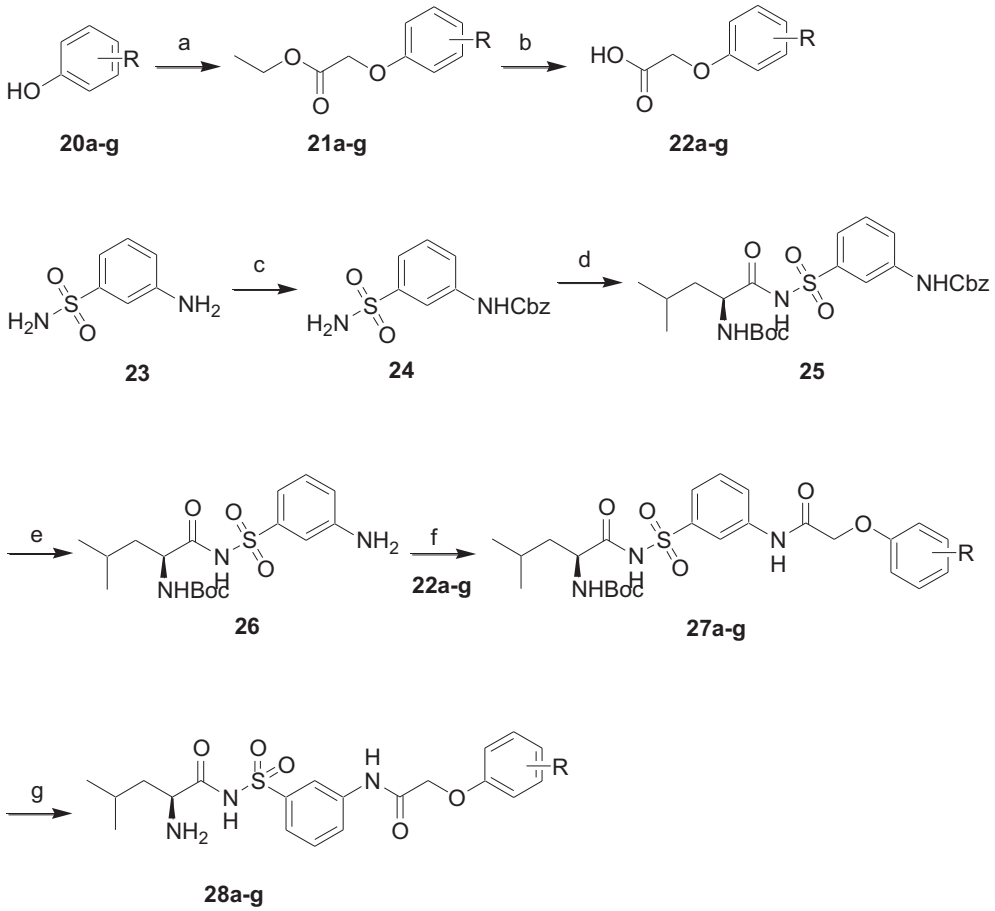


Scheme 2. Synthesis of N-(3-sulfamophenyl)amide derivatives. Reagent and conditions: (a) ethyl bromoacetate, K_2CO_3, DMF, 70 °C; (b) 1 M NaOH, dioxane; (c) CbzCl, $NaHCO_3$, H_2O/acetone; (d) *N*-Boc-leucine, EDCI, DMAP, DCM; (e) 10% Pd/C, H_2, MeOH; (f) oxalyl chloride, DCM; (g) HCl gas, EtOAc.

*5.1.6. 2-(4′-Bromo-2′-chlorophenoxy)-N-((4'-((N-carbobenzoxy-L-phenyl alanyl)amino)sulfonyl)phenyl)acetamide (**7**)*

Compound **7** (61.6 mg, 15.2% yield) was prepared from **5** (200 mg, 0.48 mmol) and L-Cbz-phenylalanine (135 mg, 0.71 mmol) following the general procedure. The crude product was washed with DCM to give **7** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.41 (s, 1H), 10.66 (s, 1H), 7.89 (d, J = 8.9 Hz, 2H), 7.83 (d, J = 8.9 Hz, 2H), 7.71 (d, J = 2.4 Hz, 1H), 7.61 (d, J = 8.2 Hz, 1H), 7.48 (dd, J = 8.9, 2.4 Hz, 1H), 7.34–7.28 (m, 3H), 7.26–7.16 (m, 7H), 7.07 (d, J = 8.9 Hz, 1H), 4.93 (s, 2H), 4.92 (s, 2H), 4.32–4.19 (m, 1H), 2.91 (dd, J = 13.5, 4.0 Hz, 1H), 2.61 (dd, J = 13.4, 10.8 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.29, 166.99, 156.30, 153.47, 143.46, 137.54, 137.21, 133.63, 132.53, 131.41, 129.66, 129.43, 128.76, 128.54, 128.23, 128.05, 126.94, 123.26, 119.37, 116.29, 112.90, 68.09, 65.91, 56.88, 36.73. Mp: 191–192 °C. HRMS: $[M+Na]^+$ calcd $[C_{31}H_{27}BrClN_3O_7S + Na]^+$ 722.0339, found 722.0497. HPLC: Gradient B, 97.0% purity, 14.1 min.

*5.1.7. 2-(4′-Bromo-2′-chlorophenoxy)-N-(4'-((N-tert-butoxycarbonyl-L- phenylalanylamino)sulfonyl)phenyl)acetamide (**8**)*

Compound **8** (170 mg, 54.3% yield) was prepared from **5** (197 mg, 0.47 mmol) and L-Boc-phenylalanine (249 mg, 0.95 mmol) following the general procedure. The crude product was purified with column chromatography and to give **8** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.31 (s, 1H), 10.64 (s, 1H), 7.85 (d, J = 8.8 Hz, 2H), 7.79 (d, J = 8.7 Hz, 2H), 7.70 (d, J = 2.2 Hz, 1H), 7.47 (dd, J = 8.9, 2.2 Hz, 1H), 7.28–7.13 (m, 5H), 7.07–7.03 (m, 2H), 4.91 (s, 2H), 4.21–4.06 (m, 1H), 2.84–2.79 (m, 1H), 2.60–2.54 (m, 1H), 1.24 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.96, 155.72, 153.46, 137.66, 132.53, 131.41, 129.67, 129.34, 128.50, 126.86, 123.25, 119.28, 116.27, 112.90, 78.82, 68.11, 56.62, 36.57, 28.52. Mp: 218–219 °C. HRMS: $[M+Na]^+$ calcd $[C_{28}H_{29}BrClN_3O_7S + Na]^+$ 688.0496, found 688.0521. HPLC: Gradient A, 100.0% purity, 17.2 min.

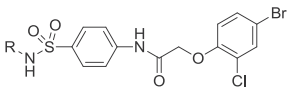
*5.1.8. 2-(4′-Bromo-2′-chlorophenoxy)-N-(4'-((L-phenylalanylamino) sulfonyl)phenyl)acetamide (**9**)*

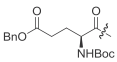
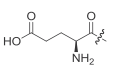
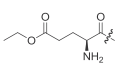
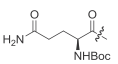
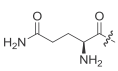
Compound **8** (102 mg, 0.15 mmol) was treated with HCl gas in DCM to give **9** as a white solid (80 mg, 86.7% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.87 (s, 1H), 10.88 (s, 1H), 8.25 (s, 3H), 7.87 (d, J = 9.0 Hz, 2H), 7.83 (d, J = 8.9 Hz, 2H), 7.71 (d, J = 2.4 Hz, 1H), 7.48 (dd, J = 8.8, 2.4 Hz, 1H), 7.21–7.20 (m, 3H), 7.07 (d, J = 8.9 Hz, 1H), 7.04–7.01 (m, 2H), 4.95 (s, 2H), 4.03 (s, 1H), 3.04 (dd, J = 13.6, 5.4 Hz, 1H), 2.96–2.91 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.05, 167.10, 153.48, 143.77, 134.29, 132.54, 131.39, 130.01, 129.69, 128.92, 127.69, 123.24, 119.43, 116.22, 112.87, 68.05, 54.20, 36.37. Mp: 224–225 °C. HRMS: $[M+H]^+$ calcd $[C_{23}H_{21}BrClN_3O_5S + H]^+$ 566.0152, found 566.0173. HPLC: Gradient A, 100.0% purity, 11.2 min.

*5.1.9. N-(4'-(((5"-Benzoxy-2"-(S)-((tert-butoxycarbonyl)amino)-5"-oxo) pentanoicylamino)sulfonyl)phenyl)-2-(4′-bromo-2′-chlorophenoxy) acetamide (**10**)*

Compound **10** (224 mg, 84.8% yield) was prepared from **5** (150 mg, 0.36 mmol) and (*S*)-5-benzyloxy-2-(*tert*-butoxycarbonyl) amino- 5-oxopentanoic acid (242 mg, 0.72 mmol) following the

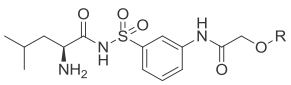
Table 1
The inhibitory activity of phenoxy-N-(4-sulfamoylphenyl)acetamides against *Tb*LeuRS.



Compd	R	*Tb*LeuRS IC_{50}(μM)
5		191.1
6		251.1
7		115.5
8		280.8
9		>1000
10		98
11		428.2
12		>1000
13		685.3
14		285.4
15		346.1
16		>1000
17		110.4
18		403.6
19		38.4

general procedure. The crude product was purified with column chromatography to give **10** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.17 (s, 1H), 10.63 (s, 1H), 7.85 (d, J = 8.9 Hz, 2H), 7.79 (d, J = 8.7 Hz, 2H), 7.70 (d, J = 2.4 Hz, 1H), 7.46 (dd, J = 8.9, 2.4 Hz, 1H), 7.40–7.27 (m, 5H), 7.10 (d, J = 7.1 Hz, 1H), 7.04 (d, J = 8.9 Hz, 1H), 5.06 (s, 2H), 4.90 (s, 2H), 3.96–3.87 (m, 1H), 2.32–2.30 (m, 1H), 2.27–2.22 (m, 1H), 1.82–1.75 (m, 1H), 1.72–1.64 (m, 1H), 1.31 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.21, 166.88, 155.69, 153.41, 136.56, 132.48, 131.36, 129.25, 129.21, 128.85, 128.42, 128.27, 123.19, 119.13, 119.10, 116.22, 112.84, 109.98, 78.86, 68.01, 65.95, 30.42, 28.53. Mp: 164–165 °C. HRMS: $[M+Na]^+$ calcd $[C_{31}H_{33}BrClN_3O_9S + Na]^+$ 760.0707, found 760.0701. HPLC: Gradient A, 100.0% purity, 17.7 min.

Table 2
The inhibitory activity of phenoxy-N-(3-sulfamoylphenyl)acetamides against *Tb*LeuRS.



Compd	R	TbLeuRS IC_{50}(μM)
28a		5.51
28b		5.69
28c		6.81
28d		2.68
28e		3.58
28f		7.28
28g		0.70

*5.1.10. (S)-4-Amino-5-(4-(2-(4′-bromo-2′-chlorophenoxy) acetamido)phenylsulfonamido)-5-oxopentanoic acid hydrochloride (**11**)*

To the solution of **10** (80 mg, 0.108 mmol) in 5 mL MeOH was added dropwise NaOH (8.6 mg, 0.215 mmol) in 2 mL water with ice bath. After stirring for 3 h under ice bath, the mixture was diluted with water, acidified to pH 2–3 and extracted with ethyl acetate (20 mL × 3). The organic layer was dried over anhydrous Na_2SO_4 and evaporated to give 84 mg residue. The residue was treated with HCl gas in DCM and purified by preparative HPLC to give **11** as a white solid (9 mg, 14.3% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.68 (s, 1H), 8.13 (s, 3H), 7.88 (d, J = 8.3 Hz, 2H), 7.78 (d, J = 8.6 Hz, 2H), 7.71 (d, J = 2.4 Hz, 1H), 7.48 (dd, J = 8.9, 2.3 Hz, 1H), 7.05 (d, J = 8.8 Hz, 1H), 4.91 (s, 2H), 3.78 (s, 1H), 2.37–2.26 (m, 2H), 1.98–1.87 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.30, 168.43, 167.03, 153.43, 143.80, 132.49, 131.37, 129.61, 123.18, 119.39, 116.20, 112.83, 67.96, 52.46, 29.31, 26.11. Mp: 167–168 °C. HRMS: $[M+H]^+$ calcd $[C_{19}H_{19}BrClN_3O_7S + H]^+$ 547.9894, found 547.9903. HPLC: Gradient B, 95.6% purity, 10.0 min.

*5.1.11. (S)-Ethyl-4-amino-5-(4-(2-(4′-bromo-2′-chlorophenoxy) acetamido) phenylsulfonamido)-5-oxopentanoate hydrochloride (**12**)*

To the solution of **11** (202 mg, 0.27 mmol) in 10 mL MeOH was added dropwise NaOH (49.8 mg, 1.24 mmol) in 0.5 mL water with ice bath. After stirring for 3 h with ice bath, the mixture was diluted with water, acidified with 1 M HCl to pH 2–3 and extracted with ethyl acetate (20 mL × 3). The organic layer was dried over anhydrous Na_2SO_4 and evaporated to give 117 mg residue. The residue was treated with HCl gas in ethyl acetate subsequently. After the reaction was completed, the solvent was removed. The residue was purified by preparative HPLC to give **12** as a white solid (11 mg, 6.6% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.93 (s, 1H), 8.32 (s, 3H), 7.89 (d, J = 8.9 Hz, 2H), 7.83 (d, J = 8.8 Hz, 2H), 7.70 (d, J = 2.3 Hz, 1H), 7.47 (dd, J = 8.8, 2.3 Hz, 1H), 7.06 (d, J = 8.9 Hz, 1H), 4.94 (s, 2H), 4.03 (q, J = 7.0 Hz, 2H), 3.86 (s, 1H), 2.37–2.25 (m, 1H), 2.21–2.11 (m,

Please cite this article as: W. Xin et al., Design and synthesis of α-phenoxy-*N*-sulfonylphenyl acetamides as *Trypanosoma brucei* Leucyl-tRNA synthetase inhibitors, European Journal of Medicinal Chemistry, https://doi.org/10.1016/j.ejmech.2019.111827

1H), 1.96 (d, $J = 7.7$ Hz, 2H), 1.16 (t, $J = 7.1$ Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.56, 166.36, 153.47, 140.75, 140.26, 132.47, 131.37, 128.32, 123.20, 118.55, 116.22, 112.80, 68.08, 60.39, 54.24, 29.72, 26.89, 14.52. Mp: 115–117 °C. HRMS: $[M+Na]^+$ calcd $[C_{21}H_{23}BrClN_3O_7S + Na]^+$ 598.0027, found 598.0025. HPLC: Gradient B, 97.7% purity, 10.0 min.

*5.1.12. (S)-Tert-butyl(5-amino-1-(4-(2-(4′-bromo-2′-chlorophenoxy)acetamido) phenylsulfonamido)-1,5-dioxopentan-2-yl)carbamate (**13**)*

Compound **13** (141 mg, 30.4% yield) was prepared from **5** (300 mg, 0.71 mmol) and (*S*)-5-amino-2-((*tert*-butoxycarbonyl) amino)-5- oxopentanoic acid (264 mg, 1.07 mmol) following the general procedure. The residue was dissolved in DCM and washed with 0.5 M NaOH solution (20 mL). The aqueous phase was acidified and filtered to give a white solid. The solid was recrystallized from DCM/MeOH/hexane to give **13** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.12 (s, 1H), 10.63 (s, 1H), 7.86 (d, $J = 8.3$ Hz, 2H), 7.80 (d, $J = 8.4$ Hz, 2H), 7.71 (d, $J = 2.3$ Hz, 1H), 7.48 (dd, $J = 8.7$, 2.2 Hz, 1H), 7.24 (s, 1H), 7.08–7.02 (m, 2H), 6.77 (s, 1H), 4.92 (s, 2H), 3.91–3.79 (m, 1H), 2.12–1.93 (m, 2H), 1.80–1.65 (m, 1H), 1.64–1.51 (m, 1H), 1.33 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.89, 166.85, 153.48, 132.51, 131.42, 129.10, 123.24, 119.11, 116.28, 112.87, 78.70, 68.09, 55.06, 31.84, 28.59. Mp: 171–172 °C. HRMS: $[M+Na]^+$ calcd $[C_{24}H_{28}BrClN_4O_8S + Na]^+$ 669.0398, found 669.0383. HPLC: Gradient A, 100.0% purity, 13.2 min.

*5.1.13. 2-(4′-Bromo-2′-chlorophenoxy)-N-(4′-((L-glutaminylamino) sulfonyl)phenyl)acetamide (**14**)*

Compound **13** (97 mg, 0.15 mmol) was treated with HCl gas in DCM to give **14** as a white solid (69 mg, 78.7% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.76 (s, 1H), 8.22 (s, 3H), 7.91 (d, $J = 8.8$ Hz, 2H), 7.82 (d, $J = 8.5$ Hz, 2H), 7.70 (d, $J = 2.3$ Hz, 1H), 7.48 (dd, $J = 8.6$, 2.3 Hz, 2H), 7.07 (d, $J = 8.9$ Hz, 1H), 6.98 (s, 1H), 4.93 (s, 2H), 3.80 (s, 1H), 2.15 (t, $J = 7.7$ Hz, 2H), 1.94–1.85 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 177.52, 173.52, 167.04, 153.48, 143.58, 133.50, 132.53, 131.41, 129.58, 123.22, 119.41, 116.24, 112.86, 68.01, 56.08, 52.97, 30.69. Mp: 165–166 °C. HRMS: $[M+H]^+$ calcd $[C_{19}H_{20}BrClN_4O_6S + H]^+$ 547.0053, found 547.0046. HPLC: Gradient A, 97.3% purity, 8.4 min.

*5.1.14. 2-(4′-Bromo-2′-chlorophenoxy)-N-(4′-(((N-tert-butoxycarbonyl- L-isoleucyl)amino)sulfonyl)phenyl)acetamide (**15**)*

Compound **15** (369 mg, 97.1% yield) was prepared from **5** (249 mg, 0.59 mmol) and L-Boc-isoleucine (278 mg, 1.18 mmol) following the general procedure. The crude product was purified with column chromatography to give **15** as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.29 (s, 1H), 8.81 (s, 1H), 8.06 (d, $J = 8.7$ Hz, 2H), 7.79 (d, $J = 8.8$ Hz, 2H), 7.61 (d, $J = 2.3$ Hz, 1H), 7.41 (dd, $J = 8.7$, 2.3 Hz, 1H), 6.86 (d, $J = 8.8$ Hz, 1H), 4.87 (d, $J = 7.6$ Hz, 1H), 4.66 (s, 2H), 3.88 (s, 1H), 1.64–1.59 (m, 2H), 1.43 (s, 9H), 1.16–1.02 (m, 1H), 0.95–0.80 (m, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.93, 166.94, 155.90, 153.47, 143.38, 133.72, 132.52, 131.40, 129.39, 123.25, 119.14, 116.28, 112.89, 78.78, 68.09, 59.30, 36.21, 28.58, 24.72, 15.40, 11.08. Mp: 187–188 °C. HRMS: $[M+Na]^+$ calcd $[C_{25}H_{31}BrClN_3O_7S + Na]^+$ 654.0653, found 654.0667. HPLC: Gradient A, 95.2% purity, 17.0 min.

*5.1.15. 2-(4′-Bromo-2′-chlorophenoxy)-N-(4′-((L-isoleucylamino) sulfonyl)phenyl)acetamide (**16**)*

Compound **15** (170 mg, 0.27 mmol) was treated with HCl gas in DCM to give **16** as a white solid (148 mg, 96.8% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.81 (s, 1H), 10.92–10.83 (m, 1H), 8.22 (s, 3H), 7.90 (d, $J = 8.8$ Hz, 2H), 7.83 (d, $J = 8.6$ Hz, 2H), 7.70 (d, $J = 2.3$ Hz, 1H), 7.47 (dd, $J = 8.8$, 2.3 Hz, 1H), 7.06 (d, $J = 8.9$ Hz, 1H), 4.99–4.88 (m, 2H), 3.67 (s, 1H), 1.83–1.78 (m, 1H), 1.26–1.18 (m, 1H), 1.06–0.95 (m, 1H), 0.85–0.69 (m, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.54, 167.03, 153.48, 143.54, 132.52, 131.39, 129.56, 123.22, 119.24, 116.23, 112.84, 68.01, 57.40, 36.58, 24.19, 14.97, 11.63. Mp: 259–260 °C. HRMS: $[M+H]^+$ calcd $[C_{20}H_{23}BrClN_3O_5S + H]^+$ 532.0308, found 532.0319. HPLC: Gradient A, 100.0% purity, 10.8 min.

*5.1.16. 2-(4′-Bromo-2′-chlorophenoxy)-N-(4′-(((N-carbobenzoxyl-L-leucyl)amino)sulfonyl)phenyl)acetamide (**17**)*

Compound **17** (173 mg, quantitative yield) was prepared from **5** (100 mg, 0.24 mmol) and L-Cbz-leucine (126 mg, 0.48 mmol) following the general procedure. The crude product was purified by column chromatography to give **17** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.23 (s, 1H), 10.61 (s, 1H), 7.85 (d, $J = 8.8$ Hz, 2H), 7.79 (d, $J = 9.2$ Hz, 2H), 7.70 (d, $J = 2.4$ Hz, 1H), 7.47 (dd, $J = 8.9$, 2.4 Hz, 1H), 7.34–7.29 (m, 5H), 7.05 (d, $J = 8.9$ Hz, 1H), 4.97 (s, 2H), 4.91 (s, 2H), 4.03 (s, 1H), 1.60–1.49 (m, 1H), 1.39–1.29 (m, 2H), 0.81 (dd, $J = 8.5$, 6.8 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.90, 156.37, 153.48, 137.33, 132.51, 131.41, 129.25, 128.79, 128.23, 123.25, 119.22, 116.29, 112.88, 68.07, 65.95, 53.91, 24.67, 23.42, 21.51. Mp: 71–72 °C. HRMS: $[M+Na]^+$ calcd $[C_{28}H_{29}BrClN_3O_7S + Na]^+$ 688.0496, found 688.0499. HPLC: Gradient A, 97.3% purity, 17.3 min.

*5.1.17. 2-(4′-Bromo-2′-chlorophenoxy)-N-(4′-(((N-tert-butoxycarbonyl-L- leucyl)amino)sulfonyl)phenyl)acetamide (**18**)*

Compound **18** (382 mg, quantitative yield) was prepared from **5** (252 mg, 0.6 mmol) and L-Boc-leucine (278 mg, 1.2 mmol) following the general procedure. The crude product was purified with column chromatography to give **18** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.60 (s, 1H), 8.80 (s, 1H), 8.05 (d, $J = 8.7$ Hz, 2H), 7.78 (d, $J = 8.8$ Hz, 2H), 7.60 (d, $J = 2.3$ Hz, 1H), 7.41 (dd, $J = 8.7$, 2.2 Hz, 1H), 6.86 (d, $J = 8.7$ Hz, 1H), 4.74 (s, 1H), 4.66 (s, 2H), 4.08–3.94 (m, 1H), 1.69–1.58 (m, 1H), 1.44 (s, 9H), 0.88 (dd, $J = 10.9$, 6.1 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.92, 155.80, 153.46, 132.52, 131.40, 129.25, 123.25, 119.19, 116.27, 112.89, 78.70, 68.09, 53.53, 28.58, 24.68, 23.33, 21.64. Mp: 85–86 °C. HRMS: $[M+Na]^+$ calcd $[C_{25}H_{31}BrClN_3O_7S + Na]^+$ 654.0653, found 654.0655. HPLC: Gradient A, 96.2% purity, 17.1 min.

*5.1.18. 2-(4′-Bromo-2′-chlorophenoxy)-N-(4′-((L-leucylamino) sulfonyl)phenyl)acetamide (**19**)*

Compound **18** (193 mg, 0.30 mmol) was treated with HCl gas in DCM to give **19** as a white solid (139 mg, 80.6% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.85 (s, 1H), 10.88 (s, 1H), 8.23 (s, 3H), 7.90 (d, $J = 8.9$ Hz, 2H), 7.83 (d, $J = 8.8$ Hz, 2H), 7.70 (d, $J = 2.4$ Hz, 1H), 7.47 (dd, $J = 8.9$, 2.4 Hz, 1H), 7.06 (d, $J = 8.9$ Hz, 1H), 4.94 (s, 2H), 3.79 (s, 1H), 1.57 (dt, $J = 12.6$, 6.4 Hz, 1H), 1.49 (t, $J = 6.8$ Hz, 2H), 0.82 (dd, $J = 6.2$, 2.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.46, 167.06, 153.48, 143.67, 133.40, 132.52, 131.39, 129.58, 123.22, 119.37, 116.23, 112.84, 68.01, 51.83, 23.87, 23.10, 22.04. Mp: 263–264 °C. HRMS: $[M+H]^+$ calcd $[C_{20}H_{23}BrClN_3O_5S + H]^+$ 532.0308, found 532.0314. HPLC: Gradient A, 100.0% purity, 10.9 min.

*5.1.19. General procedure for the synthesis of **22a-g***

Anhydrous K_2CO_3 (1.1 eq) was added to a solution of **20a-g** (1.0 eq.) and ethyl 2-bromoacetate (1.0 eq.) in DMF. After stirring overnight at 70 °C, the mixture was poured into water and extracted with ethyl acetate. The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, and concentrated to obtain **21a-g** as a colorless oil without further purification.

1 M NaOH was added dropwise to a solution of **21a-g** in dioxane at 0 °C. After stirring at r.t. for 1 h, the dioxane was evaporated and the residue was washed with ethyl acetate. Then the water layer was acidified with 1 M HCl to pH = 3 and extracted with ethyl acetate. The combined organic layer was washed with brine (50 mL),

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dried over anhydrous Na_2SO_4, and concentrated or directly filtered the solid to give **22a-g** as a white solid.

*5.1.20. 2-Phenoxyacetic acid (**22a**)*

Compound **22a** (635 mg, 73.4% yield for two steps) was prepared from phenol (532 mg, 5.7 mmol) and ethyl 2-bromoacetate (967 mg, 5.9 mmol) following the general procedure. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.95 (br. s, 1H), 7.27–7.31 (m, 2H), 6.89–6.97 (m, 3H), 4.66 (s, 2H).

*5.1.21. 2-(2-Chlorophenoxy)acetic acid (**22b**)*

Compound **22b** (1.2 g, 87.3% yield) was prepared from 2-chlorophenol (945 mg, 7.39 mmol) and ethyl 2-bromoacetate (1256 mg, 7.7 mmol) following the general procedure. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.07 (br. s, 1H), 7.43 (dd, J = 7.6, 1.2 Hz, 1H), 7.25–7.29 (m, 1H), 6.94–7.03 (m, 2H), 4.80 (s, 2H).

*5.1.22. 2-(4-Nitrophenoxy)acetic acid (**22c**)*

Compound **22c** (446 mg, 37.6% yield) was prepared from 4-nitrophenol (836 mg, 6.02 mmol) and ethyl 2-bromoacetate (1023 mg, 6.2 mmol) following the general procedure. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.19 (br. s, 1H), 8.20 (d, J = 9.2 Hz, 2H), 7.14 (d, J = 9.2 Hz, 2H), 4.88 (s, 2H).

*5.1.23. 2-(3-Methoxyphenoxy)acetic acid (**22d**)*

Compound **22d** (890 mg, 75.9% yield) was prepared from 3-methoxyphenol (798 mg, 6.45 mmol) and ethyl 2-bromoacetate (1094 mg, 6.6 mmol) following the general procedure. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.98 (br. s, 1H), 7.17 (t, J = 8.0 Hz, 1H), 6.47–6.55 (m, 3H), 4.65 (s, 2H), 3.72 (s, 3H).

*5.1.24. 2-(4-Methylphenoxy)acetic acid (**22e**)*

Compound **22e** (1.2 g, 78.7% yield) was prepared from 4-methylphenol (1038 mg, 8.37 mmol) and ethyl 2-bromoacetate (1423 mg, 8.6 mmol) following the general procedure. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.90 (br. s, 1H), 7.08 (d, J = 8.0 Hz, 2H), 6.79 (d, J = 8.4 Hz, 2H), 4.62 (s, 2H), 3.72 (s, 3H).

*5.1.25. 2-(4-Phenylphenoxy)acetic acid (**22f**)*

Compound **22f** (759 mg, 69.0% yield) was prepared from 4-phenylphenol (820 mg, 4.8 mmol) and ethyl 2-bromoacetate (819 mg, 5.0 mmol) following the general procedure. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.59 (d, J = 7.6 Hz, 2H), 7.52 (d, J = 8.8 Hz, 2H), 7.42 (t, J = 7.6 Hz, 2H), 7.29 (t, J = 7.6 Hz, 1H), 6.87 (d, J = 7.6 Hz, 2H), 4.11 (s, 2H).

*5.1.26. 2-(3-Phenylphenoxy)acetic acid (**22g**)*

Compound **22g** (768 mg, 70.3% yield) was prepared from 3-phenylphenol (815 mg, 4.8 mmol) and ethyl 2-bromoacetate (815 mg, 5.0 mmol) following the general procedure. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.65 (d, J = 8.0 Hz, 2H), 7.46 (t, J = 7.6 Hz, 2H), 7.35–7.39 (m, 2H), 7.25 (d, J = 7.6 Hz, 1H), 7.16 (s, 1H), 6.91 (dd, J = 8.4, 2.4 Hz, 1H), 4.76 (s, 2H).

*5.1.27. Benzyl (3-sulfamoylphenyl)carbamate(**24**)*

CbzCl (10 mL, 114.4 mmol) was added dropwise to a solution of **23** (10.0 g, 58.0 mmol) and sodium bicarbonate (7.4 g, 88.0 mmol) in H_2O/acetone (40/150 mL) at 0 °C, then slowly warmed to r.t. After stirred overnight, acetone was removed by rotary evaporation and dried. The white residue was triturated with H_2O and filtered. The dried precipitate was washed with cold DCM and ether to afford **24** (8.4 g, 95.9% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.08 (s, 1H), 8.06 (s, 1H), 7.5–7.55 (m, 1H), 7.51–7.34 (m, 7H), 7.32 (s, 2H), 5.18 (s, 2H).

*5.1.28. Benzyl (3-(((N-Boc-L-leucyl)amino)sulfonyl)phenyl) carbamate (**25**)*

EDCI (2.00 g, 10.5 mmol) and DMAP (1.27 g, 10.5 mmol) were added to a solution of **24** (1.07 g, 3.5 mmol) and L-Boc-leucine (1.61 g, 7.0 mmol) in DCM at 0 °C, then the mixture was slowly warmed to r.t. and stirred overnight. The mixture was washed with 1 M HCl for three times and brine for once, dried over anhydrous Na_2SO_4 and concentrated to give the residue which can be purified by chromatography on silica gel, to afford **25** (1.1 g, 60.6% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.54 (s, 1H), 7.92 (s, 2H), 7.72 (d, J = 6.9 Hz, 1H), 7.46 (t, J = 8.1 Hz, 1H), 7.40–7.34 (m, 5H), 7.06 (s, 1H), 5.21 (s, 2H), 4.78 (s, 1H), 4.06 (s, 1H), 1.41 (s, 9H), 1.33–1.24 (m, 3H), 0.91–0.83 (m, 6H).

*5.1.29. N-(3-((Boc-L-leucyl)amino)sulfonyl)phenylamine(**26**)*

To a solution of **25** (4.2 g, 8.0 mmol) in methanol (100 mL), 10% Pd/C was added under N_2 atmosphere. After 5 h, 30 mL DCM was added to quench the reaction. Then filtered to afford **26** (3.0 g, quantitative yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.96 (br. s, 1H), 7.09–7.21 (m, 2H), 6.95–6.99 (m, 2H), 6.79 (d, J = 8.0 Hz, 1H), 5.63 (s, 2H), 3.96–4.02 (m, 1H), 1.48–1.53 (m, 1H), 1.34 (s, 9H), 1.90 (s, 2H), 0.81 (t, J = 7.6 Hz, 6H).

*5.1.30. General procedures for the synthesis **28a-g***

To a solution of the acid (**22a-g**) (1.2 eq.) in DCM, oxalyl chloride and 1 drop DMF were added dropwise at 0 °C. After 3 h, DCM was removed by rotary evaporation. The resulting acyl chloride dissolved in THF and pyridine was added dropwise into a solution of **26** (1.0 eq.) in THF at 0 °C, then slowly warmed to r.t. and continued stirring for 5–6 h. 1 M HCl was added and extracted with ethyl acetate for three times. The organic layer was combined, washed with brine for once, dried over anhydrous Na_2SO_4 and concentrated to afford compounds **27a-g**. To the solution of **27a-g** in ethyl acetate, HCl gas was added for 0.5 h, and the resulting solid was filtered to obtain desired compounds **28a-g**.

*5.1.31. 2-Phenoxy-N-((3-((L-leucyl)amino)sulfonyl)phenyl) acetamide (**28a**)*

Compound **28a** (51.0 mg, 28.4% yield) was prepared from **22a** (71 mg, 0.48 mmol) and **26** (151 mg, 0.41 mmol) following the general procedure. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.96 (s, 1H), 10.76 (s, 1H), 8.44 (s, 1H), 8.34 (s, 3H), 7.95 (d, J = 8.1 Hz, 1H), 7.68–7.64 (m, 1H), 7.59 (t, J = 8.0 Hz, 1H), 7.36–7.29 (m, 2H), 7.02 (d, J = 8.7 Hz, 2H), 6.98 (d, J = 7.3 Hz, 1H), 4.77 (s, 2H), 3.84 (s, 1H), 1.67–1.45 (m, 3H), 0.83 (dd, J = 6.1, 2.8 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.96, 167.20, 157.71, 139.25, 139.03, 129.54, 129.44, 124.34, 122.52, 121.13, 118.15, 114.58, 66.84, 51.27, 23.32, 22.50, 21.55. Mp: 239–242 °C. HRMS: $[M+H]^+$ calcd $[C_{20}H_{25}N_3O_5S + H]^+$ 420.1593, found 420.1582. HPLC: Gradient A, 97.6% purity, 7.6 min.

*5.1.32. 2-(2-Chlorophenoxy)-N-((3-((L-leucyl)amino)sulfonyl) phenyl) acetamide (**28b**)*

Compound **28b** (90 mg, 37.4% yield) was prepared from **22b** (109 mg, 0.6 mmol) and **26** (188 mg, 0.5 mmol) following the general procedure. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.01 (s, 1H), 11.05 (s, 1H), 8.43 (t, J = 1.7 Hz, 1H), 8.40 (s, 2H), 7.91 (d, J = 8.2 Hz, 1H), 7.69–7.64 (m, 1H), 7.59 (t, J = 8.0 Hz, 1H), 7.46 (dd, J = 7.9, 1.5 Hz, 1H), 7.32–7.26 (m, 1H), 7.12 (dd, J = 8.3, 1.2 Hz, 1H), 6.99 (td, J = 7.8, 1.2 Hz, 1H), 4.94 (s, 2H), 3.86 (s, 1H), 1.62–1.53 (m, 3H), 0.83 (dd, J = 6.0, 4.5 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.51, 167.10, 153.88, 139.85, 139.58, 130.58, 130.16, 128.70, 124.62, 123.05, 122.59, 121.90, 118.43, 114.46, 67.90, 51.83, 23.85, 23.04, 22.05. Mp: 257–259 °C. HRMS: $[M+H]^+$ calcd $[C_{20}H_{24}ClN_3O_5S + H]^+$ 454.1203, found 454.1189. HPLC: Gradient A, 97.0% purity, 8.1 min.

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*5.1.33. 2-(4-Nitrophenoxy)-N-((3-((L-leucyl)amino)sulfonyl) phenyl)acetamide (**28c**)*

Compound **28c** (79 mg, 33.8% yield) was prepared from **22c** (110 mg, 0.55 mmol) and **26** (179 mg, 0.46 mmol) following the general procedure. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.07 (s, 1H), 11.11–10.86 (m, 1H), 8.42 (s, 1H), 8.33 (s, 2H), 8.24 (d, J = 9.2 Hz, 2H), 7.97–7.90 (m, 1H), 7.67 (d, J = 8.1 Hz, 1H), 7.60 (t, J = 8.0 Hz, 1H), 7.27–7.16 (m, 2H), 5.00 (s, 2H), 3.84 (s, 1H), 1.65–1.45 (m, 3H), 0.83 (t, J = 5.6 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.52, 166.73, 163.59, 141.75, 139.45, 130.15, 126.28, 124.81, 123.13, 118.64, 115.80, 67.63, 51.84, 23.85, 23.04, 22.02. Mp: 259–261 °C. HRMS: $[M+H]^+$ calcd $[C_{20}H_{24}N_4O_7S + H]^+$ 465.1444, found 465.1427. HPLC: Gradient A, 96.5% purity, 7.7 min.

*5.1.34. 2-(3-Methoxyphenoxy)-N-((3-((L-leucyl)amino)sulfonyl) phenyl)acetamide (**28d**)*

Compound **28d** (79 mg, 36.9% yield) was prepared from **22d** (89 mg, 0.5 mmol) and **26** (158 mg, 0.41 mmol) following the general procedure. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.61 (s, 1H), 8.43 (s, 1H), 8.21 (s, 3H), 7.93 (d, J = 7.7 Hz, 1H), 7.66 (d, J = 7.9 Hz, 1H), 7.59 (t, J = 7.9 Hz, 1H), 7.24–7.19 (m, 1H), 6.62–6.55 (m, 3H), 4.74 (s, 2H), 3.79 (s, 1H), 3.75 (s, 3H), 1.63–1.51 (m, 3H), 0.84 (dd, J = 6.1, 2.9 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.52, 167.66, 160.93, 159.44, 139.52, 130.49, 130.07, 118.72, 116.18, 115.07, 107.35, 107.23, 101.61, 67.51, 55.63, 51.85, 23.86, 23.04, 22.04. Mp: 231–233 °C. HRMS: $[M+H]^+$ calcd $[C_{21}H_{27}N_3O_6S + H]^+$ 450.1699, found 450.1680. HPLC: Gradient A, 96.7% purity, 7.7 min.

*5.1.35. 2-(4-Methoxyphenoxy)-N-((3-((L-leucyl)amino)sulfonyl) phenyl)acetamide (**28e**)*

Compound **28e** (51 mg, 24.1% yield) was prepared from **22e** (95 mg, 0.52 mmol) and **26** (167 mg, 0.43 mmol) following the general procedure. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.64 (s, 1H), 8.44 (s, 1H), 8.29 (s, 3H), 7.93 (t, J = 8.3 Hz, 1H), 7.66 (d, J = 7.8 Hz, 1H), 7.59 (t, J = 8.0 Hz, 1H), 6.99–6.94 (m, 2H), 6.89 (d, J = 9.1 Hz, 2H), 4.69 (s, 2H), 3.84–3.76 (m, 1H), 3.71 (s, 3H), 1.64–1.42 (m, 3H), 0.84 (dd, J = 6.0, 2.7 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.52, 167.96, 154.32, 152.25, 139.56, 130.05, 124.89, 123.05, 118.71, 116.17, 115.07, 68.19, 55.85, 51.82, 23.86, 23.04, 22.07. Mp: 258–260 °C. HRMS: $[M+H]^+$ calcd $[C_{21}H_{27}N_3O_6S + H]^+$ 450.1699, found 450.1685. HPLC: Gradient A, 96.5% purity, 7.6 min.

*5.1.36. 2-([1,1′-Biphenyl]-4-yloxy)-N-((3-((L-leucyl)amino) sulfonyl)phenyl)acetamide (**28f**)*

28f (73 mg, 34.4% yield) was prepared from **22f** (109 mg, 0.48 mmol) and **26** (153 mg, 0.41 mmol) following the general procedure. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.72 (s, 1H), 8.45 (s, 1H), 8.26 (s, 3H), 7.95 (d, J = 8.1 Hz, 1H), 7.70–7.57 (m, 6H), 7.44 (t, J = 7.7 Hz, 2H), 7.32 (t, J = 7.3 Hz, 1H), 7.14–7.09 (m, 2H), 4.83 (s, 2H), 3.81 (s, 1H), 1.65–1.46 (m, 3H), 0.83 (dd, J = 6.1, 3.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.53, 167.69, 157.92, 140.17, 139.57, 133.74, 130.10, 129.34, 128.25, 127.30, 126.70, 124.86, 123.07, 118.69, 115.61, 67.51, 51.83, 23.86, 23.05, 22.06. Mp: 266–268 °C. HRMS: $[M+H]^+$ calcd $[C_{26}H_{29}N_3O_5S + H]^+$ 496.1906, found 496.1910. HPLC: Gradient A, 98.4% purity, 9.4 min.

*5.1.37. 2-([1,1′-Biphenyl]-3-yloxy)-N-((3-((L-leucyl)amino) sulfonyl)phenyl)acetamide (**28g**)*

Compound **28g** (110 mg, 40.1% yield) was prepared from **22g** (141 mg, 0.62 mmol) and **26** (198 mg, 0.52 mmol) following the general procedure. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.85 (s, 1H), 8.47 (s, 1H), 8.39 (s, 3H), 7.99 (d, J = 8.1 Hz, 1H), 7.68 (d, J = 7.7 Hz, 3H), 7.60 (t, J = 8.0 Hz, 1H), 7.47 (t, J = 7.6 Hz, 2H), 7.43–7.36 (m, 2H), 7.30 (dd, J = 10.2, 4.9 Hz, 2H), 7.03 (dd, J = 8.1, 2.0 Hz, 1H), 4.88 (s, 2H), 3.86 (s, 1H), 1.65–1.50 (m, 3H), 0.82 (dd, J = 6.0, 3.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.52, 167.75, 158.75, 142.18, 140.33, 139.54, 130.51, 130.09, 129.40, 128.13, 127.22, 124.92, 123.11, 120.20, 118.75, 114.17, 113.61, 67.54, 51.84, 23.85, 23.03, 22.06. Mp: 235–237 °C. HRMS: $[M+H]^+$ calcd $[C_{26}H_{29}N_3O_5S + H]^+$ 496.1906, found 496.1891. HPLC: Gradient A, 99.3% purity, 9.5 min.

5.2. TbLeuRS assay [16]

Radioactivity was measured using a scintillation counter (Beckman LS 6500). Brewer's yeast tRNA was purchased from Roche.

T. brucei LeuRS was cloned and expressed using pET21a vector in *E. coli* strain BL21 (DE3)-RIPL. The concentration of LeuRS with the CPM value of 1000 was defined as 1 activity unit (1 U).

LeuRS inhibition IC_{50} measurement was performed in 70 μL reaction mixtures containing 50 mM HEPES-KOH (pH 7.8), 5 mM $MgCl_2$ and 45 mM KCl, 1 mM DTT, 0.02% (w/v) BSA, 0.4 mg/mL brewer's yeast tRNA (Roche), 1 U *T. brucei* LeuRS, 3 μCi/mL [^{14}C] leucine (318 mCi/mmol, PerkinElmer), ddH_2O, and compounds at different concentrations. The mixture was pre-incubated at 37 °C without ATP for 20 min, then added 4 mM ATP at 37 °C for 15 min. Then three 20 μL aliquots were spotted on 3 mm filter paper (Whatman), washed 3 times in 5% trichloroacetic acid and 3 times in alcohol. Filter papers were then dried in oven under 85 °C for 20 min and the precipitated [^{14}C] leucine tRNA^{Leu} were quantified by liquid scintillation counting using a Beckman Coulter LS 6500 liquid scintillation counter. Data was averaged to generate an IC_{50} value using GraphPad 5 for each test compound.

A 70 μL reaction system containing 50 mM HEPES-KOH (pH 7.8), 5 mM $MgCl_2$, 45 mM KCl, 1 mM DTT, 0.02% (w/v) BSA, 0.4 mg/mL tRNA (Roche), 0.1 μL DMSO, 3 μCi/mL [^{14}C] leucine, *T. brucei* LeuRS at 37 °C, then 4 mM ATP induced reaction for 20 min [^{14}C] leucine was activated and transferred to the tRNA^{Leu}. Then it was quantified by liquid scintillation counting using a Beckman Coulter LS 6500 liquid scintillation counter.

5.3. In vitro T. brucei assay

All *in vitro* anti-parasite assays were conducted with the bloodstream-form *Trypanosoma brucei brucei* 221 strain. Parasites were cultured in T-25 vented cap flasks and kept in humidified incubators at 37 °C and 5% CO_2. The parasite culture media was complete HMI-11 medium [18]. To ensure log growth phase, trypanosomes were sub-cultured at appropriate dilutions every 2–3 days. Log phase cultures were diluted 1:10 in HMI-11 and 10 μL was counted using hemocytometer to determine parasite concentration. Parasites were diluted to 2×10^5/mL in HMI-11 to generate a 2-fold working concentration for assay. Compounds to be tested were serially diluted in DMSO, and 0.5 μL added to 49.5 μL HMI-11 in triplicate 96-well plates. Parasites from the diluted stock were added to each well (50 μL) to give a final concentration of 1.0×10^5/mL parasites in 0.4% for DMSO. Trypanosomes were incubated with compounds for 72 h at 37 °C with 5% CO_2. Resazurin (20 μL of 12.5 mg/mL stock) from Sigma-Aldrich was added to each well and plates were incubated for an additional 5 h. Assay plates were read using a plate reader (Thermo Varioskan Flash) at an excitation wavelength of 544 nm and emission of 590 nm. Triplicate data points were averaged to generate sigmoidal dose response curve and determine IC_{50} values using GraphPad Prism 5.0. IC_{50} values were measured in triplicate with an error range of ±0.2 μM. Suramin and pentamidine are used as positive control and typical average IC_{50} values are 0.007 μg/mL (0.005 μM) and 0.009 μg/mL (0.026 μM), respectively.

Please cite this article as: W. Xin et al., Design and synthesis of α-phenoxy-*N*-sulfonylphenyl acetamides as *Trypanosoma brucei* Leucyl-tRNA synthetase inhibitors, European Journal of Medicinal Chemistry, https://doi.org/10.1016/j.ejmech.2019.111827

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank National Key Research and Development Program of China (2017YFA0505200), National Science Foundation of China (81573264 and 81222042), and E-Institutes of Shanghai Universities (EISU) Chemical Biology Division for financial support of this work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2019.111827.

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