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Design and synthesis of *N*-(3-sulfamoylphenyl)amides as *Trypanosoma brucei* leucyl-tRNA synthetase inhibitors

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ABSTRACT

The protozoan parasite *Trypanosoma brucei* (*T. brucei*) causes human African trypanosomiasis (HAT), which is a fatal and neglected disease in the tropic areas, and new treatments are urgently needed. Leucyl-tRNA synthetase (LeuRS) is an attractive target for the development of antimicrobial agents. In this work, starting from the hit compound thiourea ZCL539, we designed and synthesized a series of amides as effective *T. brucei* LeuRS (*Tb*LeuRS) synthetic site inhibitors. The most potent compounds **74** and **91** showed IC_{50} of 0.24 and 0.25 μM, which were about 700-fold more potent than the starting hit compound. The structure-activity relationship was also discussed. These compounds provided a new scaffold and lead compounds for further development of antitrypanosomal agents.

1. Introduction

Human African trypanosomiasis (HAT) is a fatal disease caused by the protozoan *Trypanosoma brucei* [1–3], which is a neglected disease and mostly affects the underdeveloped regions of Africa. Transmitted by bite of tsetse fly, the parasite *Trypanosoma brucei* (*T. brucei*) enters human bloodstream and lymphatic system, proliferate and enter the central nervous system, and eventually causes death if not treated. Drugs for the treatment of HAT are limited. For a long time only five drugs were available [4,5], and these drugs have serious problems such as low efficacy, toxic side effects, complex administration, and risk of drug resistance [6,7]. Thus, new drugs for the treatment of HAT are in urgent need.

Aminoacyl-tRNA synthetases (aaRSs) play critical roles in protein synthesis by catalyzing the attachment of cognate amino acids to their corresponding tRNA [8]. Inhibition of aaRSs would cause disruption of protein synthesis in microbial and lead to antimicrobial effect. In the past, aaRSs have been studied as antibacterial and antifungal targets [9–11]. In recent years, it has also been reported as antiparasitic targets [12,13].

Our group paid attention to leucyl-tRNA synthetase (LeuRS) and has previously reported benzoxaborole-based inhibitors of *Trypanosoma brucei* LeuRS (*Tb*LeuRS) targeting the editing site [14] and *N*-(4-sulfamoylphenyl)thiourea inhibitors targeting the synthetic site [15]. In this work, in an attempt to replace the thiourea and its adjacent acetoxyl group with a single amide group we designed and synthesized a new series of *Tb*LeuRS inhibitors. Compound **74** (*N*-(3-(*N*-L-Leucylsulfamoyl) phenyl-4-methoxybenzamide) was the most potent compound with IC_{50} value of 0.24 μM, which was about 700-fold more potent than the starting hit compound. The structure-activity relationship was also discussed. This work provided a new scaffold and lead compounds for the development of new antitrypanosomal agents.

2. Chemistry

N-(sulfamoylphenyl)amides were synthesized following the similar procedure we previously reported [15]. Preparation of *N*-(4-leucylsulfamoylphenyl)amide derivatives was shown in Scheme 1 and Scheme 2. First, coupling of 4-aminobenzenesulfonamide (**1**) with *N*-Boc-L-leucine in the presence of EDCI and DMAP gave compound **2** with 36.2% yield. Removal of Boc by HCl gas gave compound **3** with 63.5% yield (Scheme 1).

Preparation of compounds **15**–**22** is depicted in Scheme 2. After protection with Cbz, compound **4** was reacted with *N*-Boc-L-leucine in the presence of EDCI and DMAP to give compound **5** with 93.2% yield, which underwent hydrogenation to give amine **6** with quantitative yield. Compounds **7**–**14** were prepared by reaction with acyl chlorides prepared from their corresponding acids and oxalyl chloride. Removal of Boc group with HCl gas gave the products **15**-**22** with yields ranging from 32.0% to 81.0%.

Preparation of *N*-(3-leucylsulfamoylphenyl)amide derivatives

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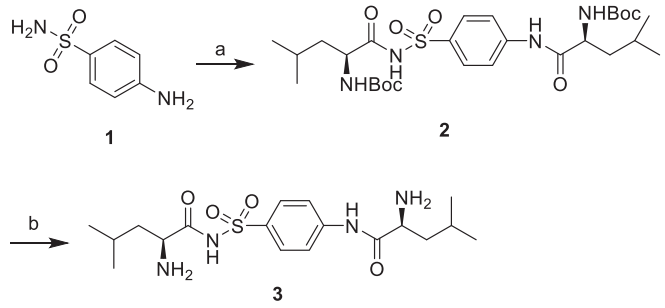
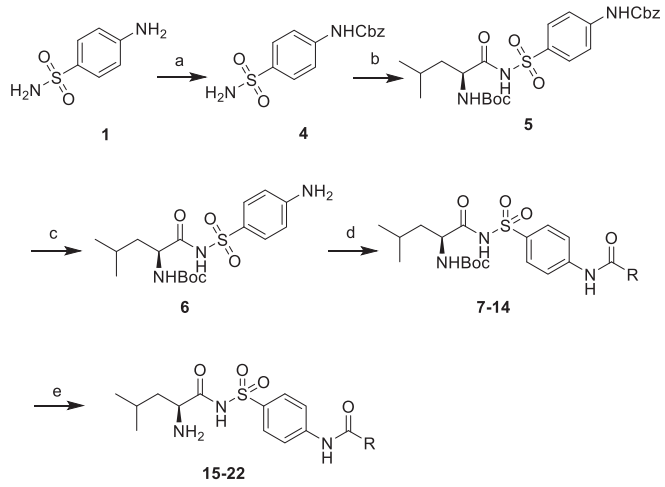
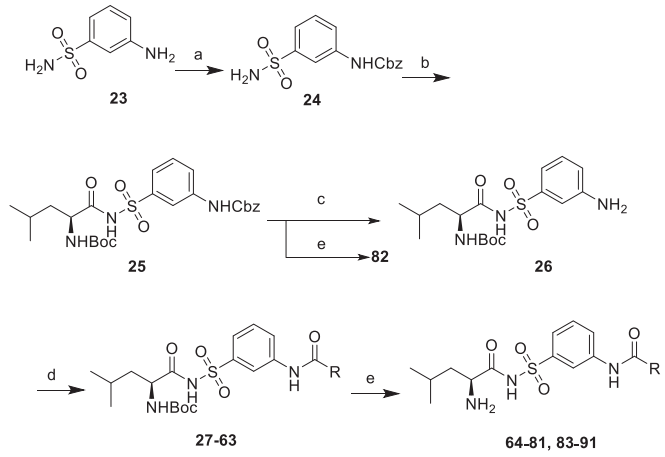


Scheme 1. Synthesis of *N*-(4-sulfamoylphenyl)amide derivative **3**. Condition and reagents: (a) *N*-Boc-L-leucine, EDCI, DMAP, DCM, 36.2%; (b) HCl (gas), EtOAc, 63.5%.



Scheme 2. Synthesis of *N*-(4-sulfamoylphenyl)amide derivatives. Condition and reagents: (a) CbzCl, $NaHCO_3$, H_2O/acetone, 84.3%; (b) *N*-Boc-L-leucine, EDCI, DMAP, DCM, 93.2%; (c) 10% Pd/C, H_2, MeOH, quantitative yield; (d) RCOOH, oxalyl chloride, DMF, DCM, then pyridine, THF; (e) HCl (gas), EtOAc, 32.0%–81.0%.



Scheme 3. Synthesis of *N*-(3-sulfamoylphenyl)amide derivatives. Condition and Reagents: (a) CbzCl, $NaHCO_3$, H_2O/acetone, quantitative yield; (b) *N*-Boc-L-Leucine, EDCI, DMAP, DCM, 50.0%; (c) Pd/C, H_2, MeOH, quantitative yield; (d) RCOOH, $SOCl_2$, then pyridine, THF; (e) HCl (gas), EtOAc, 17.0%–61.0%.

was shown in Scheme 3. First, 3-aminobenzenesulfonamide **23** was protected by Cbz group followed by reaction with *N*-Boc-L-leucine in the presence of EDCI and DMAP to afford compound **25** with 50.0% yield. Sequential removal of Cbz and Boc groups gave compound **82** with 61.0% yield. Acylation of **26** with various acyl chlorides gave compounds **27**–**63**. Eventually, removal of Boc group gave the desired products **64**-**81** and **83**-**91** with yields ranging from 17.0% to 55.0%.

3. Result and discussion

In our previous search for new *T. brucei* inhibitors, we identified a new scaffold ZCL539 (Fig. 1) with moderate activity (IC_{50} = 174 μM). Considering that the solubility of thiourea linking group in this hit compound is known to be relatively low and its toxicity also present an issue, which would limit further development of the scaffold, thus we made attempts to replace the thiourea-acetoxy linking group with a simple amide group which plays a critical role in clinically approved drugs and synthetic bioactive molecules [16]. Besides, we introduced a leucyl group at the terminal sulfonamide nitrogen with the aim to mimic the endogenous substrate. This resulted in compound **15** which showed slightly improved activity (IC_{50} = 96.0 μM) (Table 1). Introduction of a second leucyl group as in compound **3** (IC_{50} = 106.8 μM) showed slightly decreased activity, which indicated that phenyl group was more favorable than leucyl at the right terminus of the molecule. Thus, we kept the leucyl group on the left terminal sulfonamide group, and explored a number of electron withdrawing and donating substituents on the right terminal phenyl group. It turned out that both electron withdrawing and donating groups gave improved activity as exemplified by nitro compound **19** and methyl compound **21** (IC_{50} = 32.7 and 39.1 μM), which showed roughly 3-fold increase of potency as compared to compound **15**. Bromo compound **16** also gave 3-fold increase of potency. In the case of chloro compounds **17** and **18**, the *meta*-substitution was favorable, while in the case of nitro and methyl compounds **19**-**22**, the *para*-substitution was favorable. These observations indicated that both electron withdrawing and donating groups were tolerated, which suggests that electronic effect was not important in this case. Most derivatives in Table 1 showed increased activity as compared to compound **15**, however the improvement was limited.

The substrate mimicking molecule Leu-AMS (Fig. 2a) is biologically stable and is often used in cocrystallization studies for LeuRS. Its 3D geometry characteristics should provide useful insight for the optimization of LeuRS inhibitors. Thus, compound **15** was superimposed with Leu-AMS atom-by-atom using Maestro 2015 for comparison, and it was found that when the leucyl end of the two molecules were overlapped, the other end of the molecule deviated significantly from each other (Fig. 2b). While Leu-AMS has the two extending branches spaced by a single oxygen atom in the sugar ring, compound **15** with a 1,4-substitution pattern in its central phenyl ring has the two branches space by two carbon atoms. So, it implied that a 1,3-substitution pattern should make the geometry of these LeuRS inhibitors more closely resembles Leu-AMS, which might lead to higher affinity to the target, *Tb*LeuRS. This hypothesis was validated by the significant improvement of the inhibitory activity as exemplified by comparing 1,4-substituted compound **15** (IC_{50} = 96.0 μM) and its 1,3-substituted analog **64** (IC_{50} = 9.4 μM). The superimposed structures in Fig. 2c also showed that compound **64** better resembles the geometry of Leu-AMS. Thus, a series of 1,3-substitued compounds **65**-**86** was synthesized and evaluated as shown in Table 2.

As shown in Table 2, a variety of functional groups were introduced to the phenyl ring to study the SAR of the derivatives. First, halogen substitutions in compounds **65**-**67** gave inhibitory activity comparable to unsubstituted compound **64**. Next, we explored substituents with different electronic effects and found that the

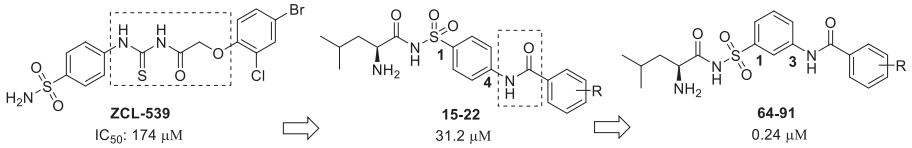
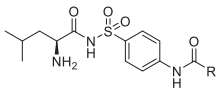


Fig. 1. Design of *N*-(sulfamoylphenyl)amide derivatives.

Table 1
Inhibitory activity of *N*-(4-sulfamoylphenyl)amide derivatives with various R groups against *Tb*LeuRS (Standard errors are within ±0.05 μM).



Compd	R	*Tb*LeuRS IC_{50}(μM)
3		106.8
15		96
16		31.2
17		115.5
18		42.8
19		32.7
20		>1000
21		39.1
22		59.7

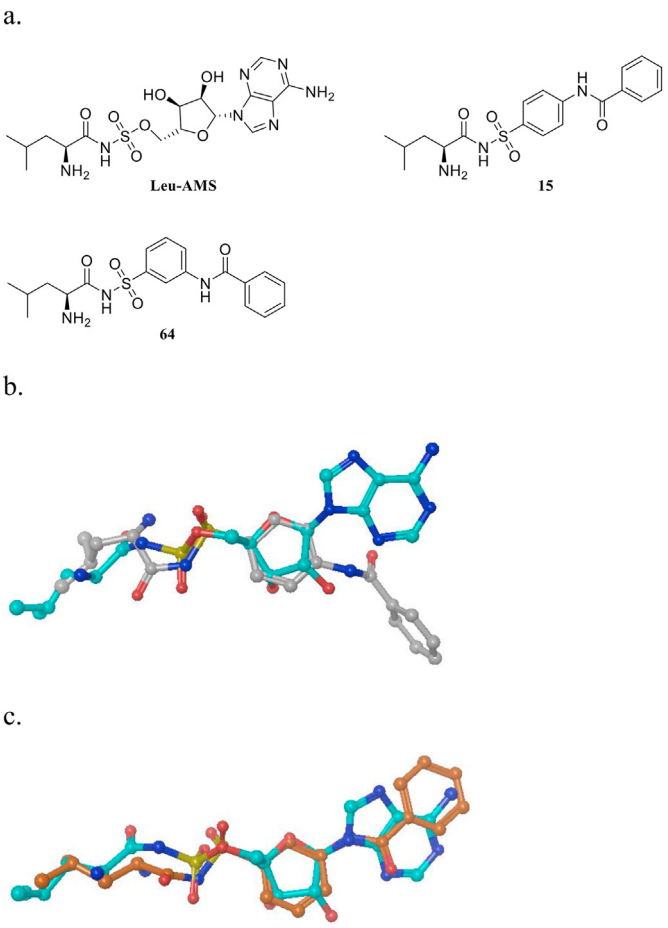
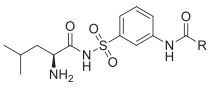


Fig. 2. a. Chemical structures of Leu-AMS and compound **15** and **64**. b. Overlay of Leu-AMS (cyan) and compound **15** (grey) atom-by-atom using Maestro 2015. c. Overlay of Leu-AMS and compound **64** (orange) atom-by-atom using Maestro 2015.

electron-withdrawing nitro group gave comparable activity (**68**-**70**, IC_{50} = 8.6–14.7 μM), methyl group (**71**-**73**, IC_{50} = 9.5–14.3 μM) also failed to improve activity, while electron-donating methoxy group (**74**-**75**, IC_{50} = 0.24 and 2.4 μM) gave nearly 40-fold increase of inhibitory activity as compared to compound **64**. Comparison of different substitution patterns in compounds **74**-**77** showed that *para*-methoxy gave the best activity. At the same time, *para*-phenoxy compound **78** also showed better activity than its *meta*-analog **79**. Replacement of the phenyl group by furan or thiophene gave activity comparable to compound **64.** Biphenyl substitution gave improved activity, especially in compound **86** which also has a methoxy substitution (IC_{50} = 1.8 μM). Those results indicated that phenyl group could be replaced by electron-rich aromatic rings.

As the activity of phenoxy derivatives **74**-**78** were superior and gave the most potent *Tb*LeuRS inhibitor (compound **74**) so far in this study, it was worthwhile to modify the phenoxy group in order to further improve the potency, for which we synthesized a number of derivatives as shown in Table 3. First, the unsubstituted OH group in compound **87** also gave good activity (IC_{50} = 0.30 μM). Next, when the substituent was a sterically bulky group such as isopropyl in compound **89**, the activity was diminished by 10-fold as compared to methoxy or OH groups, while planar group such as benzyl and linear group such ethyl were well accommodated as shown by compounds **88** and **91** (IC_{50} = 0.50 and 0.25 μM), which implied that sterically bulky substituents should be avoided. Thus, we successfully replaced the linking group in the hit compound ZCL539 from thiourea-acetoxy to a simple amide group and obtained good *Tb*LeuRS inhibitors with ~700-fold improvement of inhibitory activity from the initial hit compound. The *Tb*LeuRS inhibitors whose IC_{50} values were lower than 3 μM were also tested on the *T. brucei* 221 parasites, and among them compound **91** showed inhibition of parasite growth with an IC_{50} of 69.8 μM, while others showed no obvious inhibition. The water solubility of these compounds was tested and found to be low, thus the low *in vitro* antiparasitic activity may be attributed to their low aqueous solubility. Future optimization will be focused on their physicochemical properties in order to achieve satisfactory antiparasitic activity.

Table 2
Inhibitory activity of *N*-(3-sulfamoylphenyl)amide with different R groups against *Tb*LeuRS (Standard errors are within ±0.05 μM).



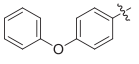
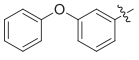
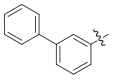
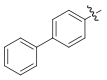
Compd	R	*Tb*LeuRS IC_{50}(μM)
64		9.4
65		6.4
66		4.9
67		5.2
68		14.7
69		8.6
70		11.5
71		14.0
72		9.5
73		14.3
74		0.24
75		2.4
76		0.66
77		1.48
78		1.3
79		8.6
80		3.7
81		6.2
82		4.0
83		3.6
84		5.8

Table 2 (*continued*)

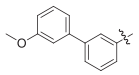
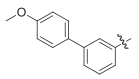
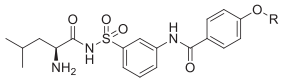
Compd	R	*Tb*LeuRS IC_{50}(μM)
85		3.6
86		1.8

Table 3
Inhibirtory activity of *N*-(3-sulfamoylphenyl)amides with different ether substituents against *Tb*LeuRS (Standard errors are within ±0.05 μM).



Compd	R	*Tb*LeuRS IC_{50}(μM)
87	**H**	0.30
88		0.50
89		3.9
90		0.80
91		0.25

4. Conclusions

Human African trypanosomiasis, a neglected and fatal tropical disease, is in urgent need of new therapeutics, especially those in new chemical classes. LeuRS, as a clinically validated antimicrobial target, should serve as a promising antitrypanosomal target. Herein, we reported the discovery of a new class of *N*-(3-sulfamoylphenyl)amide *Tb*LeuRS inhibitors. Compounds **74** and **91** showed IC_{50} of 0.24 and 0.25 μM, which were about 700-fold more potent than the hit compound ZCL539. The structure-activity relationship was also discussed. This study provided a new scaffold for further development of antitrypanosomal agents.

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). 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 = 20.0 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. (S)-2-((Tert-Butoxycarbonyl)amino)-N-(4-((N′-tert-butoxy carbonyl-L-leucylamino)sulfonyl)phenyl)-4-methylpentanamide (**2**)*

To a mixture of 4-aminobenzenesulfonamide **1** (172 mg, 1.00 mmol) and N-Boc-L-lencine (694 mg, 3.00 mmol), EDCI (1.9 g, 10.00 mmol) and DMAP (366 mg, 3.00 mmol) were added at 0 °C, then the mixture was warmed to r.t. and stirred overnight. The mixture was washed with 0.1 M HCl (20 mL × 3), 0.1 M NaOH (20 mL × 3) and brine (20 mL), dried over anhydrous Na_2SO_4 and concentrated. The residue was washed with DCM. Compound **2** was obtained as a white solid (395 mg, 36.2% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.10 (s, 1H), 10.39 (s, 1H), 7.88–7.78 (m, 4H), 7.11 (d, J = 7.6 Hz, 1H), 6.99 (d, J = 7.7 Hz, 1H), 1.63 (s, 1H), 1.51 (dd, J = 18.6, 9.8 Hz, 2H), 1.37 (s, 9H), 1.30 (s, 9H), 1.23 (s, 3H), 0.88 (dd, J = 6.3, 3.9 Hz, 6H), 0.79 (dd, J = 9.8, 6.7 Hz, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.16, 172.72, 155.99, 155.81, 129.23, 118.98, 78.69, 78.56, 54.15, 53.48, 28.11, 24.81, 24.66, 23.37, 23.31, 22.00, 21.64. Mp: 212–213 °C. HRMS: $[M+Na]^+$ calcd $[C_{28}H_{46}N_4O_8S+Na]^+$ 621.2934, found 621.2937. HPLC: gradient B, 98.0% purity, 16.3 min.

*5.1.2. (S)-2-Amino-N-(4-((L-leucylamino)sulfonyl)phenyl)-4-methyl-pentanamide (**3**)*

Compound **2** (100 mg, 0.17 mmol) was dissolved in ethyl acetate, the solution was treated with HCl gas, and **3** was obtained as a white precipitate (50 mg, 63.5% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.82 (s, 1H), 11.56 (s, 1H), 8.50 (s, 3H), 8.29 (s, 3H), 7.91 (q, J = 9.0 Hz, 4H), 4.10 (s, 1H), 3.79 (s, 1H), 1.68 (d, J = 3.8 Hz, 3H), 1.60–1.46 (m, 3H), 0.92 (t, J = 5.4 Hz, 6H), 0.82 (d, J = 6.1 Hz, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.21, 129.56, 119.64, 52.22, 24.25, 23.88, 23.09, 22.67, 22.04. Mp: 72–73 °C. HRMS: $[M+H]^+$ calcd $[C_{18}H_{30}N_4O_4S+H]^+$ 399.2066, found 399.2056. HPLC: gradient A, 97.0% purity, 5.5 min.

*5.1.3. Benzyl 4-sulfamoylphenylcarbamate (**4**)*

CbzCl (10.0 mL, 116.28 mmol) was added dropwise to a solution of **1** (10.0 g, 58.14 mmol) and $NaHCO_3$ (7.4 g, 88.10 mmol) in H_2O/acetone (40/150 mL) at 0 °C, then the mixture was slowly warmed to r.t. After the mixture was 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, **4** was obtained as white solid (15.0 g, 84.3% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.75 (d, J = 8.8 Hz, 2H), 7.63 (d, J = 8.8 Hz, 2H), 7.33–7.46 (m, 5H), 5.18 (s, 2H).

*5.1.4. Benzyl(4-(N-(N-tert-butoxycarbonyl-L-leucyl)sulfamoyl) phenyl) carbamate (**5**)*

EDCI (9.3 g, 48.51 mmol) and DMAP (5.9 g, 48.51 mmol) were added to a solution of **4** (5.0 g, 16.17 mmol) and N-Boc-L-leucine (7.5 g, 32.34 mmol) in DCM at 0 °C, the mixture was slowly warmed to r.t. and stirred overnight. The mixture was washed with 1 M HCl for three times and brine once, dried over anhydrous Na_2SO_4 and concentrated to give the residue, the residue was then purified by column chromatography to afford **5** (7.9 g, 93.2% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.07 (s, 1H), 10.28 (s, 1H), 7.81 (d, J = 8.8 Hz, 2H), 7.65 (d, J = 8.8 Hz, 2H), 7.35–7.45 (m, 5H), 6.99 (d, J = 8.0 Hz, 1H), 5.19 (s, 2H), 3.95 (br. s, 1H), 1.50 (br. s, 1H), 1.23–1.38 (m, 10H), 1.14 (s, 1H), 0.80 (dd, J = 9.2, 6.8 Hz, 6H).

*5.1.5. N-(N-Tert-butoxycarbonyl-L-leucyl)-4-aminobenzenesulfonamide (**6**)*

To a solution of **5** (4.2 g, 8.00 mmol) in methanol (100 mL), 10% Pd/C (420 mg) was added. The reaction was stirred at r.t under H_2 atomsphere. After 5 h, 30 mL DCM was added to quench the reaction and the Pd/C was removed by filtration, the solution was concentrated to gain **6** (3.2 g, 100% yield) as white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.69 (s, 1H), 7.51 (d, J = 8.4 Hz, 2H), 6.92 (d, J = 8.0 Hz, 2H), 6.58 (d, J = 8.4 Hz, 2H), 6.11 (s, 2H), 3.95 (br. s, 1H), 1.46–1.53 (m, 1H), 1.34 (s, 9H), 1.16–1.22 (m, 2H), 0.78–0.82 (m, 6H).

*5.1.6. General procedure 1 for preparation of **15**–**22***

To a solution of organic acetic acids (1.2 eq) in DCM, oxalyl chloride in DMF was added dropwise at 0 °C. After 3 h, DCM was removed by rotary evaporation. Acyl chloride dissolved in THF and pyridine was added dropwise into a solution of **6** (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, compounds **7**–**14** were obtained. Then the intermediates **7**–**14** were dissolved in ethyl acetate, treated with HCl gas for 0.5 h, the resulting precipitate was filtered to obtain the products.

*5.1.7. N-(4-(N-L-Leucylsulfamoyl)phenyl)benzamide (**15**)*

Compound **15** (25.8 mg, 32.0% yield) was prepared from **7** (73 mg, 0.19 mmol) and benzoic acid (25 mg, 0.21 mmol) following general procedure 1. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.71 (s, 1H), 8.22 (s, 3H), 8.04 (d, J = 8.8 Hz, 2H), 8.00 (d, J = 8.0 Hz, 2H), 7.95 (d, J = 8.9 Hz, 2H), 7.64 (t, J = 7.3 Hz, 1H), 7.57 (t, J = 7.4 Hz, 2H), 3.80 (s, 1H), 1.66–1.49 (m, 3H), 0.86 (dd, J = 6.3, 1.3 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.50, 166.61, 144.53, 134.72, 132.57, 129.39, 128.96, 128.38, 126.98, 120.26, 51.86, 23.87, 23.16, 21.90. Mp: 257–259 °C. HRMS: $[M+H]^+$ calcd $[C_{19}H_{23}N_3O_4S+H]^+$ 390.1488, found 390.1486. HPLC: gradient A, 98.9% purity, 7.2 min.

*5.1.8. N-(4-(N-L-Leucylsulfamoyl)phenyl)-4-bromobenzamide (**16**)*

Compound **16** (88.3 mg, 61.9% yield) was prepared from **8** (108 mg, 0.28 mmol) and 4-bromobenzoic acid (67.8 mg, 0.33 mmol) following general procedure 1. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.97 (s, 1H), 10.94 (s, 1H), 8.42 (s, 3H), 8.08 (d, J = 8.4 Hz, 2H), 8.00 (d, J = 8.0 Hz, 2H), 7.94 (d, J = 8.4 Hz, 2H), 7.75 (d, J = 8.0 Hz, 2H), 3.87 (s, 1H), 1.62–1.54 (m, 3H), 0.84 (d, J = 4.4 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.82, 165.08, 143.89, 133.16, 130.05, 128.77, 125.81, 119.86, 51.20, 23.31, 22.53, 21.54. Mp: 261–262 °C. HRMS: $[M+H]^+$ calcd $[C_{19}H_{22}BrN_3O_4S+H]^+$ 468.0592, found 468.0596. HPLC: gradient A, 98.6% purity, 8.2 min.

*5.1.9. N-(4-(N-L-Leucylsulfamoyl)phenyl)-4-chlorobenzamide (**17**)*

Compound **17** (100.6 mg, 81.0% yield) was prepared from **9** (103.8 mg, 0.27 mmol) and 4-chlorobenzoic acid (50.5 mg, 0.32 mmol) following general procedure 1. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.84 (s, 1H), 10.83 (s, 1H), 8.28 (s, 3H), 8.05 (d, J = 8.8 Hz, 2H), 8.04 (d, J = 8.4 Hz, 2H), 7.95 (d, J = 8.8 Hz, 2H), 7.62 (d, J = 8.4 Hz, 2H), 3.84 (s, 1H), 1.64–1.52 (m, 3H), 0.84 (dd, J = 6.0, 2.4 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.77, 164.97, 143.95, 136.86, 132.81, 129.85, 128.87, 128.48, 119.87, 51.25, 23.29, 22.57, 21.40. Mp: 253–255 °C. HRMS: $[M+H]^+$ calcd $[C_{19}H_{22}ClN_3O_4S+H]^+$ 424.1098, found 424.1095. HPLC: gradient A, 97.7% purity, 8.1 min.

*5.1.10. N-(4-(N-L-Leucylsulfamoyl)phenyl)-3-chlorobenzamide (**18**)*

Compound **18** (41.2 mg, 33.2% yield) was prepared from **10** (103.8 mg, 0.27 mmol) and 3-chlorobenzoic acid (50.5 mg,0.32 mmol) following general procedure 1. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.89 (s, 1H), 8.36 (s, 3H), 8.08 (d, J = 2.0 Hz, 2H), 8.05 (s, 1H), 7.97 (t, J = 9.0 Hz, 3H), 7.71 (ddd, J = 8.0, 2.0, 0.9 Hz, 1H), 7.60 (t, J = 7.9 Hz, 1H), 3.86 (s, 1H), 1.68–1.49 (m, 3H), 0.85 (dd, J = 6.3, 1.9 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.83, 164.58,

143.75, 136.09, 133.17, 131.77, 130.37, 128.80, 127.58, 126.70, 119.89, 51.21, 23.31, 22.54, 21.48. Mp: 241–242 °C. HRMS: $[M+H]^+$ calcd $[C_{19}H_{22}ClN_3O_4S+H]^+$ 424.1098, found 424.1097. HPLC: gradient A, 95.6% purity, 8.1 min.

*5.1.11. N-(4-(N-L-Leucylsulfamoyl)phenyl)-4-nitrobenzamide (**19**)*

Compound **19** (65.8 mg, 55.8% yield) was prepared from **11** (96 mg, 0.25 mmol) and 4-nitrobenzoic acid (50 mg, 0.30 mmol) following general procedure 1. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.20 (s, 1H), 8.39 (s, 3H), 8.37 (s, 2H), 8.28 (d, J = 8.8 Hz, 2H), 8.10 (d, J = 8.9 Hz, 2H), 7.98 (d, J = 8.8 Hz, 2H), 3.88 (s, 1H), 1.67–1.53 (m, 3H), 0.85 (dd, J = 6.1, 1.9 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.85, 164.44, 149.31, 143.58, 139.73, 133.14, 129.49, 128.84, 123.44, 120.02, 51.21, 23.31, 22.54, 21.49; Mp: 259–260 °C. HRMS: $[M+H]^+$ calcd $[C_{19}H_{22}N_4O_6S+H]^+$ 435.1338, found 435.1334. HPLC: gradient A, 97.1% purity, 7.7 min.

*5.1.12. N-(4-(N-L-Leucylsulfamoyl)phenyl)-3-nitrobenzamide (**20**)*

Compound **20** (84.9 mg, 65.6% yield) was prepared from **12** (105 mg, 0.27 mmol) and 3-nitrobenzoic acid (55 mg, 0.33 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.96 (s, 1H), 11.20 (s, 1H), 8.83 (s, 1H), 8.51 (d, J = 7.7 Hz, 1H), 8.47 (d, J = 8.3 Hz, 1H), 8.40 (s, 3H), 8.10 (d, J = 8.7 Hz, 2H), 7.98 (d, J = 8.7 Hz, 2H), 7.86 (t, J = 8.0 Hz, 1H), 3.87 (s, 1H), 1.67–1.54 (m, 3H), 0.86 (d, J = 5.1 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.87, 163.92, 147.66, 143.57, 135.53, 134.37, 130.16, 128.83, 126.48, 122.66, 120.07, 51.23, 23.32, 22.54, 21.49. Mp: 269–271 °C. HRMS: $[M+H]^+$ calcd $[C_{19}H_{22}N_4O_6S+H]^+$ 435.1338, found 435.1335. HPLC: gradient A, 95.6% purity, 7.7 min.

*5.1.13. N-(4-(N-L-Leucylsulfamoyl)phenyl)-4-methylbenzamide (**21**)*

Compound **21** (73.7 mg, 63.9% yield) was prepared from **13** (100 mg, 0.26 mmol) and 4-methylbenzoic acid (42.7 mg, 0.32 mmol) following general procedure 1. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.68 (s, 1H), 8.33 (s, 3H), 8.06 (d, J = 8.8 Hz, 2H), 7.94 (dd, J = 8.6, 1.8 Hz, 4H), 7.36 (d, J = 8.1 Hz, 2H), 3.84 (s, 1H), 2.40 (s, 3H), 1.65–1.52 (m, 3H), 0.85 (dd, J = 6.3, 1.6 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.84, 165.84, 144.18, 142.11, 132.52, 131.23, 128.88, 128.74, 127.95, 119.72, 51.21, 23.33, 22.54, 21.57, 20.97. Mp: 271–273 °C. HRMS: $[M+H]^+$ calcd $[C_{20}H_{25}N_3O_4S+H]^+$ 404.1644, found 404.1641. HPLC: gradient A, 97.1% purity, 7.8 min.

*5.1.14. N-(4-(N-L-Leucylsulfamoyl)phenyl)-3-methylbenzamide (**22**)*

Compound **22** (43.6 mg, 33.8% yield) was prepared from **14** (111 mg, 0.293 mmol) and 3-methylbenzoic acid (47.8 mg, 0.35 mmol) following general procedure 1. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.74 (s, 1H), 8.38 (s, 3H), 8.07 (d, J = 8.8 Hz, 2H), 7.95 (d, J = 8.8 Hz, 2H), 7.82 (dd, J = 9.8, 4.8 Hz, 2H), 7.44 (d, J = 4.7 Hz, 2H), 3.86 (s, 1H), 2.41 (s, 3H), 1.65–1.53 (m, 3H), 0.85 (d, J = 5.6 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.81, 166.14, 144.12, 137.72, 134.11, 132.55, 128.77, 128.30, 128.26, 125.04, 119.72, 51.19, 23.32, 22.54, 20.84. Mp: 252–253 °C. HRMS: $[M+H]^+$ calcd $[C_{20}H_{25}N_3O_4S+H]^+$ 404.1644, found 404.1644. HPLC: gradient A, 95.7% purity, 7.7 min.

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

To a solution of 3-aminobenzenesulfonamide **23** (5.0 g, 29.07 mmol) and $NaHCO_3$ (3.7 g, 43.61 mmol) in 65 mL H_2O/acetone, CbzCl (5 mL) was added, the mixture was stirred overnight at r.t. under N_2 atomsphere. After the reaction, the mixture was evaporated and solid was washed by H_2O, DCM and ether to afford **24** (10.0 g, 100% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.09 (s, 1H), 8.07 (s, 1H), 7.58–7.60 (m, 1H), 7.34–7.49 (m, 9H), 5.18 (s, 2H).

*5.1.16. Benzyl(3-(N-(N-tert-butoxycarbonyl-L-leucyl)sulfamoyl) phenyl) carbamate (**25**)*

To a solution of **24** (2.0 g, 6.53 mmol) and N-Boc-L-leucine (3.0 g, 12.99 mmol), EDCI (5.0 g, 26.08 mmol), DMAP (1.6 g, 12.99 mmol) in 50 mL DCM, the mixture was stirred at r.t. overnight. Then reaction finished, wash with 1 M HCl and organic layer dried by anhydrous Na_2SO_4, evaporated to remove solvent, the residue was purified by column chromatography to afford **25** (1.65 g, 50.0% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.30 (s, 1H), 10.18 (s, 1H), 8.18 (s, 1H), 7.66 (s, 2H), 7.35–7.50 (m, 7H), 7.00 (d, J = 6.0 Hz, 1H), 5.18 (s, 2H), 3,96 (br. s, 1H), 1.49 (br. s, 1H), 1.32 (s, 9H), 1.11 (s, 2H), 0.80 (s, 6H).

*5.1.17. N-(N-Tert-butoxycarbonyl-L-leucyl)-3-aminobenzene-sulfonamide (**26**)*

To a solution of compound **25** (1.5 g, 2.89 mmol) in 30 mL methanol and 10% Pd/C (150 mg) was added. The reaction was stirred at r.t under H_2 atomsphere, after 5 h, added 30 mL DCM and filtered, concentrated to afford compound **26** (1.1 g, 100% 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, 8H), 1.90 (s, 2H), 0.81 (t, J = 7.7 Hz, 6H).

*5.1.18. General procedure 2 for preparation of **64-81, 83-91***

To a solution of organic acids (1.1 eq) in $SOCl_2$, the reaction was stirred at reflux, then finished, $SOCl_2$ was removed and 5 mL THF was added and dropwise to compound **26** (1.0 eq). Mixture was stirred at r.t overnight. Then solvent was removed, H_2O and ethyl acetate was added, organic layer was dried by anhydrous Na_2SO_4 and concentrated. Next, to a solution of compounds gained above in ethyl acetate, HCl gas was added for 0.5 h, filtered the solid to afford products.

*5.1.19. N-(3-(N-L-Leucylsulfamoyl)phenyl)benzamide (**64**)*

Compound **64** (21 mg, 25.9% yield) was prepared from **26** (73 mg, 0.19 mmol) and benzoic acid (25 mg, 0.21 mmol) following general procedure 2. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.10 (s, 1H), 10.77 (s, 1H), 8.60 (s, 1H), 8.40 (s, 3H), 8.12 (d, J = 8.1 Hz, 1H), 8.07–8.01 (m, 2H), 7.68 (d, J = 7.9 Hz, 1H), 7.62 (td, J = 7.5, 4.1 Hz, 2H), 7.56 (t, J = 7.4 Hz, 2H), 3.86 (s, 1H), 1.66–1.50 (m, 3H), 0.85 (d, J = 5.5 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.58, 166.30, 140.31, 134.66, 132.42, 129.89, 128.91, 128.34, 125.59, 122.98, 119.32, 51.77, 23.88, 23.04, 22.19. Mp: 252–253 °C. HRMS: $[M+H]^+$ calcd $[C_{19}H_{23}N_3O_4S+H]^+$ 390.1488, found 390.1483. HPLC: gradient A, 99.6% purity, 7.0 min.

*5.1.20. N-(3-(N-L-Leucylsulfamoyl)phenyl)-4-fluorobenzamide (**65**)*

Compound **65** (28 mg, 33.2% yield) was prepared from **26** (73 mg, 0.19 mmol) and 4-fluorobenzoic acid (29 mg, 0.21 mmol) following general procedure 2. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.77 (s, 1H), 8.58 (s, 1H), 8.36 (s, 3H), 8.16–8.09 (m, 3H), 7.69 (d, J = 7.9 Hz, 1H), 7.62 (t, J = 7.9 Hz, 1H), 7.40 (t, J = 8.8 Hz, 2H), 3.86 (s, 1H), 1.67–1.51 (m, 3H), 0.90–0.79 (m, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.58, 165.20, 140.22, 131.09, 129.95, 125.58, 123.06, 119.35, 116.02, 115.80, 51.87, 23.88, 23.08, 22.01. Mp: 262–264 °C. HRMS: $[M+H]^+$ calcd $[C_{19}H_{22}FN_3O_4S+H]^+$ 408.1393, found 408.1388. HPLC: gradient A, 97.3% purity, 7.3 min.

*5.1.21. N-(3-(N-L-Leucylsulfamoyl)phenyl)-4-chlorobenzamide (**66**)*

Compound **66** (30.5 mg, 35.1% yield) was prepared from **26** (73 mg, 0.19 mmol) and benzoic acid (32.5 mg, 0.21 mmol) following general procedure 2. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.82 (s, 1H), 8.58 (s, 1H), 8.35 (s, 3H), 8.11 (d, J = 8.0 Hz, 1H), 8.07 (d, J = 8.6 Hz, 2H), 7.69 (d, J = 8.0 Hz, 1H), 7.63 (t, J = 8.6 Hz, 3H), 3.86 (s, 1H), 1.68–1.47 (m, 3H), 0.85 (dd, J = 6.0, 1.6 Hz, 6H). ^{13}C NMR

(101 MHz, DMSO-d_6) δ 165.23, 140.10, 137.30, 133.39, 130.27, 129.98, 129.03, 123.72, 123.17, 119.37, 51.86, 23.87, 23.10, 21.99. Mp: 270–271 °C. HRMS: $[M+H]^+$ calcd $[C_{19}H_{22}ClN_4O_6S+H]^+$ 424.1098, found 424.1101. HPLC: gradient A, 99.3% purity, 9.3 min.

5.1.22. N-(3-(N-L-Leucylsulfamoyl)phenyl)-4-bromobenzamide (**67**)

Compound **67** (27 mg, 28.2% yield) was prepared from **26** (73 mg, 0.19 mmol) and 4-bromobenzoic acid (41 mg, 0.21 mmol) following general procedure 2. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.82 (s, 1H), 8.58 (s, 1H), 8.35 (s, 3H), 8.11 (d, J = 7.9 Hz, 1H), 7.99 (d, J = 8.5 Hz, 2H), 7.78 (d, J = 8.5 Hz, 2H), 7.69 (d, J = 8.0 Hz, 1H), 7.63 (t, J = 8.0 Hz, 1H), 3.86 (s, 1H), 1.68–1.50 (m, 3H), 0.92–0.79 (m, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.36, 140.09, 133.77, 131.97, 130.42, 129.97, 126.27, 125.59, 123.17, 119.40, 51.89, 23.88, 23.09, 22.01. Mp: 277–278 °C. HRMS: $[M+H]^+$ calcd $[C_{19}H_{22}BrN_3O_4S+H]^+$ 468.0593, found 468.0597. HPLC: gradient A, 98.9% purity, 8.3 min.

5.1.23. N-(3-(N-L-Leucylsulfamoyl)phenyl)-4-nitrobenzamide (**68**)

Compound **68** (25.7 mg, 35.7% yield) was prepared from **26** (59 mg, 0.15 mmol) and 4-nitrobenzoic acid (28 mg, 0.17 mmol) following general procedure 2. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.12 (s, 1H), 8.60 (t, J = 1.8 Hz, 1H), 8.39 (dd, J = 6.9, 2.0 Hz, 5H), 8.30–8.25 (m, 2H), 8.14 (d, J = 8.8 Hz, 1H), 7.75–7.70 (m, 1H), 7.65 (t, J = 8.0 Hz, 1H), 3.87 (s, 1H), 1.68–1.51 (m, 3H), 0.85 (dd, J = 6.1, 1.6 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.60, 164.71, 149.83, 140.30, 139.84, 130.00, 124.06, 123.52, 119.51, 51.82, 23.88, 23.09, 22.02. Mp: 271–273 °C. HRMS: $[M+H]^+$ calcd $[C_{19}H_{22}N_4O_6S+H]^+$ 435.1338, found 435.1336. HPLC: gradient A, 99.9% purity, 8.7 min.

5.1.24. N-(3-(N-L-Leucylsulfamoyl)phenyl)-3-nitrobenzamide (**69**)

Compound **69** (23.2 mg, 32.2% yield) was prepared from **26** (59 mg, 0.15 mmol) and 3-nitrobenzoic acid (28 mg, 0.17 mmol) following general procedure 2. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.17 (s, 1H), 8.86 (t, J = 1.9 Hz, 1H), 8.59 (t, J = 1.8 Hz, 1H), 8.52 (dd, J = 7.9, 1.3 Hz, 1H), 8.47 (dd, J = 2.3, 0.9 Hz, 1H), 8.45–8.37 (m, 3H), 8.21–8.15 (m, 1H), 7.86 (t, J = 8.0 Hz, 1H), 7.76–7.70 (m, 1H), 7.66 (t, J = 8.0 Hz, 1H), 3.90 (s, 1H), 1.68–1.56 (m, 3H), 0.85 (dd, J = 6.1, 1.8 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.56, 164.18, 148.23, 139.80, 136.04, 134.84, 130.74, 130.10, 126.98, 125.82, 123.47, 123.11, 119.59, 51.78, 23.88, 23.07, 22.07. Mp: 268–269 °C. HRMS: $[M+H]^+$ calcd $[C_{19}H_{22}N_4O_6S+H]^+$ 435.1338, found 435.1328. HPLC: gradient A, 99.5% purity, 8.6 min.

5.1.25. N-(3-(N-L-Leucylsulfamoyl)phenyl)-2-nitrobenzamide (**70**)

Compound **70** (27.3 mg, 37.8% yield) was prepared from **26** (59 mg, 0.15 mmol) and 2-nitrobenzoic acid (28 mg, 0.17 mmol) following general procedure 2. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.20 (s, 1H), 8.47 (s, 1H), 8.40 (s, 3H), 8.18 (d, J = 8.2 Hz, 1H), 7.95–7.87 (m, 2H), 7.85–7.77 (m, 2H), 7.72 (d, J = 8.0 Hz, 1H), 7.64 (t, J = 8.0 Hz, 1H), 3.86 (s, 1H), 1.69–1.53 (m, 3H), 0.92–0.81 (m, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.60, 165.04, 146.87, 139.88, 134.69, 132.52, 131.78, 130.27, 129.81, 124.85, 123.50, 118.61, 51.84, 23.88, 23.07, 22.03. Mp: 244–246 °C. HRMS: $[M+H]^+$ calcd $[C_{19}H_{22}N_4O_6S+H]^+$ 435.1338, found 435.1338. HPLC: gradient A, 99.8% purity, 7.8 min.

5.1.26. N-(3-(N-L-Leucylsulfamoyl)phenyl)-4-methylbenzamide (**71**)

Compound **71** (30.9 mg, 36.8% yield) was prepared from **26** (73 mg, 0.19 mmol) and 4-methylbenzoic acid (28 mg, 0.21 mmol) following general procedure 2. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.99 (s, 1H), 10.61 (s, 1H), 8.59 (s, 1H), 8.26 (s, 3H), 8.09 (d, J = 8.2 Hz, 1H), 7.93 (d, J = 8.2 Hz, 2H), 7.67 (d, J = 8.0 Hz, 1H), 7.62 (t, J = 7.9 Hz, 1H), 7.37 (d, J = 8.1 Hz, 2H), 3.83 (s, 1H), 2.40 (s, 3H), 1.64–1.48 (m, 3H), 0.85 (dd, J = 6.1, 2.2 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.53, 166.14, 142.55, 140.40, 131.81, 129.93, 129.46, 128.32, 122.89, 119.26, 51.81, 23.87, 23.09, 22.00, 21.52. Mp: 259–260 °C. HRMS: $[M+H]^+$ calcd $[C_{20}H_{25}N_3O_4S+H]^+$ 404.1644, found 404.1640. HPLC: gradient A, 99.1% purity, 7.8 min.

5.1.27. N-(3-(N-L-Leucylsulfamoyl)phenyl)-3-methylbenzamide (**72**)

Compound **72** (29.3 mg, 35.2% yield) was prepared from **26** (73 mg, 0.19 mmol) and 3-methylbenzoic acid (28 mg, 0.21 mmol) following general procedure 2. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.65 (s, 1H), 8.58 (s, 1H), 8.08 (d, J = 8.1 Hz, 1H), 7.83 (s, 1H), 7.80 (t, J = 4.3 Hz, 1H), 7.68 (d, J = 8.0 Hz, 1H), 7.62 (t, J = 7.9 Hz, 1H), 7.44 (d, J = 5.0 Hz, 2H), 3.83 (s, 1H), 2.42 (s, 3H), 1.64–1.51 (m, 3H), 0.88–0.82 (m, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.43, 140.34, 138.27, 134.70, 133.02, 128.85, 128.71, 125.46, 122.96, 119.24, 51.83, 23.87, 23.09, 21.99, 21.43. Mp: 266–267 °C. HRMS: $[M+H]^+$ calcd $[C_{20}H_{25}N_3O_4S+H]^+$ 404.1644, found 404.1637. HPLC: gradient A, 98.3% purity, 7.7 min.

5.1.28. N-(3-(N-L-Leucylsulfamoyl)phenyl)-2-methylbenzamide (**73**)

Compound **73** (25.2 mg, 30.2% yield) was prepared from **26** (73 mg, 0.19 mmol) and 2-methylbenzoic acid (28 mg, 0.21 mmol) following general procedure 2. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.73 (s, 1H), 8.59 (s, 1H), 8.36 (s, 3H), 7.94 (d, J = 8.3 Hz, 1H), 7.68 (d, J = 7.9 Hz, 1H), 7.61 (t, J = 7.9 Hz, 1H), 7.50 (d, J = 7.6 Hz, 1H), 7.42 (t, J = 7.5 Hz, 1H), 7.33 (d, J = 7.6 Hz, 2H), 3.84 (s, 1H), 2.40 (s, 3H), 1.67–1.54 (m, 3H), 0.85 (d, J = 6.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.57, 168.68, 140.32, 139.97, 136.96, 135.88, 131.12, 130.46, 129.98, 127.79, 126.17, 123.08, 118.49, 99.99, 51.90, 23.88, 23.08, 22.04, 19.81. Mp: 248–250 °C. HRMS: $[M+H]^+$ calcd $[C_{20}H_{25}N_3O_4S+H]^+$ 404.1644, found 404.1638. HPLC: gradient A, 99.2% purity, 8.6 min.

5.1.29. N-(3-(N-L-Leucylsulfamoyl)phenyl)-4-methoxybenzamide (**74**)

Compound **74** (30.6 mg, 37.2% yield) was prepared from **26** (70 mg, 0.18 mmol) and 4-methoxybenzoic acid (30 mg, 0.20 mmol) following general procedure 2. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.54 (s, 1H), 8.58 (s, 1H), 8.29 (s, 3H), 8.09 (d, J = 7.9 Hz, 1H), 8.03 (d, J = 8.8 Hz, 2H), 7.66 (d, J = 7.9 Hz, 1H), 7.60 (t, J = 7.9 Hz, 1H), 7.09 (d, J = 8.9 Hz, 2H), 3.86 (s, 3H), 3.84–3.81 (m, 1H), 1.67–1.44 (m, 3H), 0.89–0.80 (m, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.59, 165.66, 162.69, 140.49, 130.25, 129.86, 126.72, 125.44, 122.74, 119.24, 114.18, 55.97, 51.94, 23.88, 23.11, 21.93. Mp: 266–268 °C. HRMS: $[M+H]^+$ calcd $[C_{20}H_{25}N_3O_5S+H]^+$ 420.1593, found 420.1587. HPLC: gradient A, 99.3% purity, 7.2 min.

5.1.30. N-(3-(N-L-Leucylsulfamoyl)phenyl)-3-methoxybenzamide (**75**)

Compound **75** (23.2 mg, 28.2% yield) was prepared from **26** (70 mg, 0.18 mmol) and 3-methoxybenzoic acid (30 mg, 0.20 mmol) following general procedure 2. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.73 (s, 1H), 8.59 (t, J = 1.8 Hz, 1H), 8.39 (s, 3H), 8.13 (d, J = 8.6 Hz, 1H), 7.71–7.66 (m, 1H), 7.62 (dd, J = 11.3, 4.4 Hz, 2H), 7.59–7.57 (m, 1H), 7.47 (t, J = 7.9 Hz, 1H), 7.21–7.17 (m, 1H), 3.87 (s, 1H), 3.86 (s, 3H), 1.67–1.45 (m, 3H), 0.87–0.82 (m, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.56, 166.03, 159.69, 140.24, 136.08, 130.01, 125.64, 123.02, 120.51, 119.40, 118.20, 113.52, 55.90, 51.85, 23.88, 23.08, 22.04. Mp: 257–258 °C. HRMS: $[M+H]^+$ calcd $[C_{20}H_{25}N_3O_5S+H]^+$ 420.1593, found 420.1588. HPLC: gradient A, 98.9% purity, 7.3 min.

*5.1.31. N-(3-(N-L-Leucylsulfamoyl)phenyl)-3,4-dimethoxybenzamide (**76**)*

Compound **76** (19.8 mg, 21.5% yield) was prepared from **26** (73 mg, 0.19 mmol) and 3,4-dimethoxybenzoic acid (38 mg, 0.21 mmol) following general procedure 2. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.57 (s, 1H), 8.54 (s, 1H), 8.34 (s, 3H), 8.13 (d, J = 6.6 Hz, 1H), 7.69 (d, J = 8.5 Hz, 1H), 7.63–7.59 (m, 2H), 7.09 (d, J = 8.1 Hz, 1H), 4.01 (s, 1H), 3.86 (s, 3H), 3.84 (s, 3H), 1.54 (s, 3H), 0.84 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.57, 165.66, 152.42, 148.79, 140.47, 129.83, 126.66, 125.65, 122.67, 121.90, 119.40, 111.67, 111.45, 56.20, 51.85, 23.89, 23.07, 22.07. Mp: 261–262 °C. HRMS: $[M+H]^+$ calcd $[C_{21}H_{27}N_3O_6S+H]^+$ 450.1699, found 450.1698. HPLC: gradient A, 98.1% purity, 6.9 min.

*5.1.32. N-(3-(N-L-Leucylsulfamoyl)phenyl)-3,4,5-trimethoxybenzamide (**77**)*

Compound **77** (25.3 mg, 32.7% yield) was prepared from **26** (59 mg, 0.15 mmol) and 3,4,5-trimethoxybenzoic acid (36 mg, 0.17 mmol) following general procedure 2. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.51 (s, 1H), 8.47 (s, 1H), 8.08 (s, 4H), 7.65 (d, J = 7.4 Hz, 1H), 7.61 (d, J = 8.0 Hz, 1H), 7.32 (s, 2H), 3.87 (s, 6H), 3.73 (s, 3H), 3.65–3.58 (m, 1H), 1.65–1.43 (m, 3H), 0.84 (d, J = 6.0 Hz, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 165.62, 153.15, 141.09, 140.13, 129.95, 129.72, 122.96, 119.57, 105.91, 60.63, 56.63, 52.04, 23.88, 23.16, 21.83. Mp: 242–244 °C. HRMS: $[M+H]^+$ calcd $[C_{22}H_{29}N_3O_7S+H]^+$ 480.1804, found 480.1803. HPLC: gradient A, 99.5% purity, 7.2 min.

*5.1.33. N-(3-(N-L-Leucylsulfamoyl)phenyl)-4-phenoxybenzamide (**78**)*

Compound **78** (18.7 mg, 23.5% yield) was prepared from **26** (59 mg, 0.15 mmol) and 4-phenoxybenzoic acid (36 mg, 0.17 mmol) following general procedure 2. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.62 (s, 1H), 8.57 (s, 1H), 8.24 (s, 2H), 8.11–8.03 (m, 3H), 7.67 (d, J = 8.0 Hz, 1H), 7.61 (t, J = 8.0 Hz, 1H), 7.48 (t, J = 8.0 Hz, 2H), 7.25 (t, J = 7.4 Hz, 1H), 7.12 (dd, J = 8.2, 5.0 Hz, 4H), 3.82 (s, 1H), 1.67–1.49 (m, 3H), 0.85 (d, J = 4.8 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.64, 165.51, 160.60, 155.89, 140.34, 130.78, 130.62, 129.88, 129.22, 125.43, 124.97, 122.90, 120.13, 119.29, 117.85, 51.93, 23.88, 23.10, 21.99. Mp: 268–269 °C. HRMS: $[M+H]^+$ calcd $[C_{25}H_{27}N_3O_5S+H]^+$ 482.1750, found 482.1752. HPLC: gradient A, 99.1% purity, 9.2 min.

*5.1.34. N-(3-(N-L-Leucylsulfamoyl)phenyl)-3-phenoxybenzamide (**79**)*

Compound **79** (15.1 mg, 19.5% yield) was prepared from **26** (59 mg, 0.15 mmol) and 3-phenoxybenzoic acid (36 mg, 0.17 mmol) following general procedure 2. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.69 (s, 1H), 8.55 (s, 1H), 8.24 (s, 2H), 8.06 (d, J = 8.0 Hz, 1H), 7.81 (d, J = 8.2 Hz, 1H), 7.68 (d, J = 8.0 Hz, 1H), 7.65–7.63 (m, 1H), 7.62–7.59 (m, 1H), 7.57 (d, J = 7.9 Hz, 1H), 7.45 (t, J = 7.8 Hz, 2H), 7.26 (dd, J = 8.2, 2.4 Hz, 1H), 7.21 (t, J = 7.3 Hz, 1H), 7.09 (d, J = 8.4 Hz, 2H), 3.81 (s, 1H), 1.68–1.45 (m, 3H), 0.84 (dd, J = 5.9, 3.9 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.46, 157.32, 156.69, 140.05, 136.58, 130.77, 130.70, 129.92, 124.41, 123.20, 123.16, 122.44, 119.41, 119.37, 118.12, 52.00, 23.87, 23.12, 21.88. Mp: 273–275 °C. HRMS: $[M+H]^+$ calcd $[C_{25}H_{27}N_3O_5S+H]^+$ 482.1750, found 482.1751. HPLC: gradient A, 99.8% purity, 9.1 min.

*5.1.35. N-(3-(N-L-Leucylsulfamoyl)phenyl)furan-2-carboxamide (**80**)*

Compound **80** (18.9 mg, 23.9% yield) was prepared from **26** (73 mg, 0.19 mmol) and furan-2-carboxylic acid (24 mg, 0.21 mmol) following general procedure 2. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.64 (s, 1H), 8.52 (s, 1H), 8.25 (s, 3H), 8.07 (d, J = 7.9 Hz, 1H), 7.98 (d, J = 1.0 Hz, 1H), 7.67 (d, J = 8.0 Hz, 1H), 7.60 (t, J = 7.9 Hz, 1H), 7.47 (d, J = 2.7 Hz, 1H), 6.73 (dd, J = 3.5, 1.7 Hz, 1H), 3.81 (s, 1H), 1.67–1.51 (m, 3H), 0.85 (dd, J = 6.1, 2.3 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 156.92, 147.44, 146.69, 139.70, 129.93, 125.33, 123.03, 119.25, 115.89, 112.72, 52.01, 23.88, 23.10, 21.91. Mp: 237–239 °C. HRMS: $[M+H]^+$ calcd $[C_{17}H_{21}N_3O_5S+H]^+$ 380.1280, found 380.1275. HPLC: gradient A, 96.3% purity, 6.1 min.

*5.1.36. N-(3-(N-L-Leucylsulfamoyl)phenyl)thiophene-2-carboxamide (**81**)*

Compound **81** (23 mg, 28.0% yield) was prepared from **26** (73 mg, 0.19 mmol) and thiophene-2-carboxylic acid (27 mg, 0.21 mmol) following general procedure 2. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.82 (s, 1H), 8.53 (s, 1H), 8.36 (s, 3H), 8.24 (d, J = 3.6 Hz, 1H), 8.14 (d, J = 8.1 Hz, 1H), 7.90 (d, J = 5.0 Hz, 1H), 7.68 (d, J = 7.9 Hz, 1H), 7.62 (t, J = 7.9 Hz, 1H), 7.28–7.23 (m, 1H), 3.87 (s, 1H), 1.67–1.51 (m, 3H), 0.89–0.80 (m, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.57, 160.74, 139.92, 132.98, 130.42, 130.00, 128.70, 125.46, 122.91, 119.24, 51.86, 23.88, 23.07, 22.01. Mp: 257–258 °C. HRMS: $[M+H]^+$ calcd $[C_{17}H_{21}N_3O_4S_2+H]^+$ 396.1052, found 396.1053. HPLC: gradient A, 98.7% purity, 6.8 min.

*5.1.37. Benzyl (3-(N-L-leucylsulfamoyl)phenyl)carbamate (**82**)*

Compound **82** (50 mg, 61.0% yield) was prepared from **25** (100 mg, 0.18 mmol) in the presence of HCl gas. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.25 (s, 1H), 8.34 (s, 3H), 8.25 (s, 1H), 7.70 (d, J = 7.7 Hz, 1H), 7.59–7.52 (m, 2H), 7.47–7.32 (m, 5H), 5.19 (s, 2H), 3.83 (s, 1H), 1.68–1.46 (m, 3H), 0.83 (d, J = 6.1 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 153.80, 140.28, 136.84, 130.08, 128.96, 128.62, 128.60, 121.95, 117.06, 66.52, 51.87, 23.85, 23.06, 22.00. Mp: 252–253 °C. HRMS: $[M+H]^+$ calcd $[C_{20}H_{25}N_3O_5S+H]^+$ 420.1593, found 420.1587. HPLC: gradient A, 98.0% purity, 8.2 min.

*5.1.38. N-(3-(N-L-Leucylsulfamoyl)phenyl)-(1,1′-biphenyl)-3-carboxamide (**83**)*

Compound **83** (52 mg, 55.0% yield) was prepared from **26** (73 mg, 0.19 mmol) and (1,1′-biphenyl)-3-carboxylic acid (41 mg, 0.21 mmol) following general procedure 2. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.81 (s, 1H), 8.59 (s, 1H), 8.29 (s, 4H), 8.14 (d, J = 8.1 Hz, 1H), 8.00 (d, J = 7.9 Hz, 1H), 7.92 (d, J = 7.9 Hz, 1H), 7.83–7.77 (m, 2H), 7.72–7.67 (m, 1H), 7.67–7.59 (m, 2H), 7.53 (t, J = 7.6 Hz, 2H), 7.44 (t, J = 7.3 Hz, 1H), 3.85 (s, 1H), 1.68–1.46 (m, 3H), 0.86 (d, J = 5.7 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.60, 166.23, 140.83, 140.28, 139.90, 135.38, 130.63, 129.84, 129.52, 128.38, 127.46, 126.46, 125.63, 123.06, 119.40, 51.84, 23.89, 23.10, 22.03. Mp: 258–259 °C. HRMS: $[M+H]^+$ calcd $[C_{25}H_{27}N_3O_4S+H]^+$ 466.1801, found 466.1810. HPLC: gradient A, 99.8% purity, 10.8 min.

*5.1.39. N-(3-(N-L-Leucylsulfamoyl)phenyl)-(1,1′-biphenyl)-4-carboxamide (**84**)*

Compound **84** (50 mg, 53.0% yield) was prepared from **26** (73 mg, 0.19 mmol) and (1,1′-biphenyl)-4-carboxylic acid (41 mg, 0.21 mmol) following general procedure 2. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.73 (s, 1H), 8.62 (s, 1H), 8.25 (s, 3H), 8.12 (t, J = 7.2 Hz, 3H), 7.87 (d, J = 8.5 Hz, 2H), 7.81–7.76 (m, 2H), 7.69 (d, J = 8.0 Hz, 1H), 7.63 (t, J = 7.9 Hz, 1H), 7.53 (t, J = 7.5 Hz, 2H), 7.45 (t, J = 7.3 Hz, 1H), 3.83 (s, 1H), 1.67–1.52 (m, 3H), 0.86 (d, J = 5.4 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.56, 165.95, 143.94, 140.35, 139.47, 133.41, 129.97, 129.57, 129.03, 128.72, 127.42, 127.13, 125.60, 123.03, 119.32, 51.82, 23.88, 23.09, 22.04. Mp: 279–280 °C. HRMS: $[M+H]^+$ calcd $[C_{25}H_{27}N_3O_4S+H]^+$ 466.1801, found 466.1799. HPLC: gradient A, 99.3% purity, 10.8 min.

*5.1.40. N-(3-(N-L-Leucylsulfamoyl)phenyl)-3′-methoxy-(1,1′-biphenyl)-3-carboxamide (**85**)*

Compound **85** (28 mg, 25.2% yield) was prepared from **26**

(80 mg, 0.21 mmol) and 3′-methoxy-(1,1′-biphenyl)-3-carboxylic acid (52 mg, 0.23 mmol) following general procedure 2. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.13 (s, 1H), 10.95 (s, 1H), 8.63 (s, 1H), 8.46 (s, 3H), 8.33 (s, 1H), 8.20 (d, J = 8.1 Hz, 1H), 8.02 (d, J = 7.8 Hz, 1H), 7.92 (d, J = 7.7 Hz, 1H), 7.71 (d, J = 7.9 Hz, 1H), 7.64 (t, J = 7.9 Hz, 2H), 7.43 (t, J = 8.1 Hz, 1H), 7.39–7.34 (m, 2H), 7.02–6.97 (m, 1H), 3.91 (s, 1H), 3.86 (s, 3H), 1.69–1.52 (m, 3H), 0.86 (d, J = 5.9 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.57, 166.23, 160.32, 141.43, 140.71, 140.32, 135.33, 130.64, 129.93, 129.61, 127.65, 126.56, 125.71, 123.03, 119.76, 119.45, 113.96, 113.02, 55.77, 51.84, 23.90, 23.07, 22.13. Mp: 232–235 °C. HRMS: $[M+H]^+$ calcd $[C_{26}H_{29}N_3O_5S+H]^+$ 496.1906, found 496.1916. HPLC: gradient A, 99.6% purity, 9.1 min.

5.1.41. N-(3-(N-L-Leucylsulfamoyl)phenyl)-4′-methoxy-(1,1′-biphenyl)-3-carboxamide (**86**)

Compound **86** (32 mg, 28.8% yield) was prepared from **26** (80 mg, 0.21 mmol) and 4′-methoxy-(1,1′-biphenyl)-3-carboxylic acid (52 mg, 0.23 mmol) following general procedure 2. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.84 (s, 1H), 8.61 (s, 1H), 8.37 (s, 3H), 8.25 (s, 1H), 8.16 (d, J = 8.1 Hz, 1H), 7.94 (d, J = 7.9 Hz, 1H), 7.87 (d, J = 7.9 Hz, 1H), 7.78–7.73 (m, 2H), 7.72–7.68 (m, 1H), 7.62 (dt, J = 11.1, 7.9 Hz, 2H), 7.12–7.06 (m, 2H), 3.88 (s, 1H), 3.83 (s, 3H), 1.68–1.54 (m, 3H), 0.86 (d, J = 5.9 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.33, 159.73, 140.49, 140.30, 135.34, 132.20, 130.07, 129.95, 129.60, 128.56, 126.72, 125.87, 123.03, 119.41, 114.94, 55.72, 51.89, 23.89, 23.10, 22.03. Mp: 257–258 °C. HRMS: $[M+H]^+$ calcd $[C_{26}H_{29}N_3O_5S+H]^+$ 496.1906, found 496.1896. HPLC: gradient A, 99.3% purity, 9.1 min.

5.1.42. N-(3-(N-L-Leucylsulfamoyl)phenyl)-4-hydroxybenzamide (**87**)

Compound **87** (15.2 mg, 18.1% yield) was prepared from **26** (73 mg, 0.19 mmol) and 4-hydroxybenzoic acid (29 mg, 0.21 mmol) following general procedure 2. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.86 (s, 1H), 10.40 (s, 1H), 10.22 (s, 1H), 8.56 (s, 1H), 8.22 (s, 3H), 8.05 (d, J = 7.9 Hz, 1H), 7.91 (d, J = 8.7 Hz, 2H), 7.64 (d, J = 7.9 Hz, 1H), 7.58 (t, J = 7.9 Hz, 1H), 6.90 (d, J = 8.7 Hz, 2H), 3.81 (s, 1H), 1.66–1.49 (m, 3H), 0.85 (dd, J = 6.1, 1.6 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.85, 140.59, 130.35, 125.34, 125.11, 122.56, 119.17, 115.48, 70.26, 23.88, 23.10, 21.98. Mp: 216–217 °C. HRMS: $[M+H]^+$ calcd $[C_{19}H_{23}N_3O_5S+H]^+$ 406.1437, found 406.1429. HPLC: gradient A, 99.9% purity, 6.2 min.

5.1.43. N-(3-(N-L-Leucylsulfamoyl)phenyl)-4-ethoxybenzamide (**88**)

Compound **88** (18.8 mg, 21.0% yield) was prepared from **26** (73 mg, 0.19 mmol) and 4-ethoxybenzoic acid (35 mg, 0.21 mmol) following general procedure 2. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.55 (s, 1H), 8.56 (s, 1H), 8.37 (s, 3H), 8.09 (d, J = 7.5 Hz, 1H), 8.01 (d, J = 8.7 Hz, 2H), 7.64 (d, J = 7.9 Hz, 1H), 7.58 (t, J = 7.9 Hz, 1H), 7.04 (d, J = 8.9 Hz, 2H), 4.11 (q, J = 7.0 Hz, 2H), 3.84 (s, 1H), 1.65–1.45 (m, 3H), 1.35 (t, J = 7.0 Hz, 3H), 0.83 (d, J = 5.2 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.67, 161.97, 130.29, 129.85, 126.53, 125.49, 122.70, 119.23, 114.54, 63.92, 51.85, 23.88, 23.07, 22.05, 15.00. Mp: 271–273 °C. HRMS: $[M+H]^+$ calcd $[C_{21}H_{27}N_3O_5S+H]^+$ 434.1750, found 434.1744. HPLC: gradient A, 99.9% purity, 7.8 min.

5.1.44. N-(3-(N-L-Leucylsulfamoyl)phenyl)-4-isopropoxybenzamide (**89**)

Compound **89** (15.6 mg, 17.0% yield) was prepared from **26** (73 mg, 0.19 mmol) and 4-isopropoxybenzoic acid (38 mg, 0.21 mmol) following general procedure 2. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.50 (s, 1H), 8.55 (s, 1H), 8.28 (s, 3H), 8.07 (d, J = 7.7 Hz, 1H), 7.98 (d, J = 8.7 Hz, 2H), 7.63 (d, J = 7.8 Hz, 1H), 7.57 (t, J = 7.9 Hz, 1H), 7.04 (d, J = 8.7 Hz, 2H), 4.75 (dt, J = 12.1, 6.1 Hz, 1H), 3.81 (s, 1H), 1.63–1.51 (m, 3H), 1.29 (d, J = 6.0 Hz, 6H), 0.83 (d, J = 5.8 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.58, 165.67, 160.99, 140.53, 130.34, 126.31, 122.68, 119.21, 115.43, 69.99, 60.22, 23.88, 23.07, 22.13, 21.23, 14.55. Mp: 267–268 °C. HRMS: $[M+H]^+$ calcd $[C_{22}H_{29}N_3O_5S+H]^+$ 448.1906, found 448.1900. HPLC: gradient A, 98.2% purity, 8.4 min.

5.1.45. N-(3-(N-L-Leucylsulfamoyl)phenyl)-4-butoxybenzamide (**90**)

Compound **90** (28 mg, 29.5% yield) was prepared from **26** (73 mg, 0.19 mmol) and 4-butoxybenzoic acid (41 mg, 0.21 mmol) following general procedure 2. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.52 (s, 1H), 8.57 (s, 1H), 8.31 (s, 3H), 8.08 (d, J = 8.0 Hz, 1H), 8.00 (d, J = 8.9 Hz, 2H), 7.64 (d, J = 8.0 Hz, 1H), 7.59 (t, J = 7.9 Hz, 1H), 7.06 (d, J = 8.9 Hz, 2H), 4.06 (t, J = 6.5 Hz, 2H), 3.84 (s, 1H), 1.76–1.67 (m, 2H), 1.63–1.51 (m, 3H), 1.43 (dt, J = 14.6, 7.4 Hz, 2H), 0.93 (t, J = 7.4 Hz, 3H), 0.83 (dd, J = 6.0, 1.7 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.51, 165.66, 162.14, 140.54, 139.71, 130.30, 129.83, 126.50, 125.50, 122.69, 119.24, 114.56, 67.96, 51.83, 31.09, 23.88, 23.06, 22.09, 19.16, 14.15. Mp: 253–255 °C. HRMS: $[M+H]^+$ calcd $[C_{23}H_{31}N_3O_5S+H]^+$ 462.2063, found 462.2062. HPLC: gradient A, 99.7% purity, 9.3 min.

5.1.46. N-(3-(N-L-Leucylsulfamoyl)phenyl)-4-benzyloxybenzamide (**91**)

Compound **91** (37 mg, 36.6% yield) was prepared from **26** (73 mg, 0.19 mmol) and 4-(benzyloxy)benzoic acid (48 mg, 0.21 mmol) following general procedure 2. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.56 (s, 1H), 8.56 (s, 1H), 8.36 (s, 3H), 8.09 (d, J = 7.7 Hz, 1H), 8.02 (d, J = 8.7 Hz, 2H), 7.64 (d, J = 8.0 Hz, 1H), 7.58 (t, J = 7.9 Hz, 1H), 7.47 (d, J = 7.3 Hz, 2H), 7.40 (t, J = 7.3 Hz, 2H), 7.34 (t, J = 7.1 Hz, 1H), 7.15 (d, J = 8.7 Hz, 2H), 5.21 (s, 2H), 3.84 (s, 1H), 1.63–1.50 (m, 3H), 0.83 (d, J = 5.5 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.55, 165.64, 161.72, 140.50, 137.09, 130.27, 129.85, 128.96, 128.46, 128.25, 126.92, 122.74, 119.23, 115.01, 69.92, 51.87, 23.88, 23.08, 22.02. Mp: 243–246 °C. HRMS: $[M+H]^+$ calcd $[C_{26}H_{29}N_3O_5S+H]^+$ 496.1906, found 496.1906. HPLC: gradient A, 98.6% purity, 9.2 min.

5.2. TbLeuRS assay [17]

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). Brewer's yeast tRNA was purchased from Roche.

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 in duplicate was averaged to generate an IC_{50} value using GraphPad 5 for each test compound and standard errors are within ±0.05 μM.

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.

Declaration of competing interest

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2021.113319.

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