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Lead optimization of selective tubulin inhibitors as anti-trypanosomal agents

Anran Zhao[a,#], Yaxin Li[a,#], Cody M. Orahoske[a], Brittny Schnur[b], Abboud Sabbagh[a], Wenjing Zhang[a], Bibo Li[b,*], Bin Su[a,*]

[a] Department of Chemistry, Center for Gene Regulation in Health and Disease, College of Sciences and Health Professions, Cleveland State University, 2121 Euclid Ave., Cleveland, OH 44115, USA

[b] Department of Biology, Geo. & Env. Sciences, Center for Gene Regulation in Health and Disease, College of Sciences and Health Professions, Cleveland State University, 2121 Euclid Ave., Cleveland, OH 44115, USA

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ABSTRACT

Previously synthesized tubulin inhibitors showed promising in vitro selectivity and activity against Human African Trypanosomiasis. Current aim is to improve the ligand efficiency and reduce overall hydrophobicity of the compounds, by lead optimization. Via combinatorial chemistry, 60 new analogs were synthesized. For biological assay *Trypanosoma brucei brucei* Lister 427 cell line were used as the parasite model and for the host model human embryonic kidney cell line HEK-293 and mouse macrophage cell line RAW 264.7 were used to test efficacy. Of the newly synthesized compounds 5, 39, 40, and 57 exhibited IC_{50}s below 5 μM inhibiting the growth of trypanosome cells and not harming the mammalian cells at equipotent concentration. Comparably, the newly synthesized compounds have a reduced amount of aromatic moieties resulting in a decrease in molecular weight. Due to importance of tubulin polymerization during protozoan life cycle its activity was assessed by western blot analyses. Our results indicated that compound 5 had a profound effect on tubulin function. A detailed structure activity relationship (SAR) was summarized that will be used to guide future lead optimization.

1. Introduction

African sleeping sickness (Human African trypanosomiasis, HAT) is a vector-borne parasitic disease that is still life threatening to the residents in sub-Saharan Africa.[1] Currently there are five drugs available for the treatment of human African trypanosomiasis, including Suramin, Pentamidine, Melarsoprol, Eflornithine, and Nifurtimox.[2] However, these drugs have many limitations: 1) highly toxic to the infected hosts; 2) Parental administration via intramuscular or intravenous injections, which is difficult to use in the epidemic area with limited medical resources; 3) narrow anti-trypanosomiasis spectrum; and 4) high cost to the patients. Overall, these drugs are not effective in the treatment of the disease, and there is an urgent need to develop more effective and inexpensive chemotherapeutic agents for the treatment of HAT.

Tubulin interfering agents have been the first line chemotherapeutic drugs for decades in cancer treatment.[3,4] Tubulin is also a critical protein of trypanosomal cells and plays an essential role during trypanosome cell division.[5,6] The fast growth rate of trypanosome cells indicates tubulin polymerization/depolymerization is essential for proliferation.[7,8] In addition, tubulin is the key protein for the locomotion of trypanosome cells, which is an essential function for them to survive.[9] Therefore, tubulin inhibitors are expected to be able to block the *T. brucei* cell division and decrease the locomotion function of the flagellum as well, which will lead to cell death.[10] Furthermore, two different tubulin inhibitors benzimidazoles and dinitroanilies also have been evaluated for HAT activity. Benzimidazoles general are used as anthelmintics and antifungal agents.[11,12] Although benzimidazoles have demonstrated selective toxicity by binding with protozoa tubulin instead of mammalian tubulin.[13] Whereas, dinitroanilines are classified as herbicides that inhibit microtubulin, and these compounds are also potent anti-protozoal agents which are effective against microtubules of *T. brucei.* cells and other parasitic cells.[14,15] Hence, these factors suggest that there are multiple advantages of tubulin inhibitor as a novel drug for the treatment of HAT.

Tubulin is a highly conserved protein within different species. However, different susceptibility to antimitotic agents are known to exist among different organisms, indicating differences in tubulin structures among different species.[16,17] Based on the differences of the colchicine binding pockets of mammalian and *T. brucei* tubulins, selective tubulin inhibitors were developed that showed great in vitro

* Corresponding authors.
E-mail addresses: b.li37@csuohio.edu (B. Li), b.su@csuohio.edu (B. Su).
# Equal contribution.

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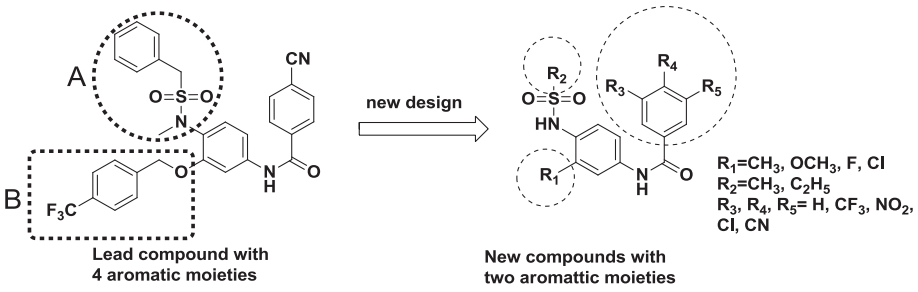


Figure 1. Core structure of the derivatives.

potency to inhibit *T. brucei* cell growth without harming mammalian cells at the similar concentrations.[18,19] Some compounds exhibited very specific inhibitory effect on *T. brucei* cell growth, with a selectivity index (IC_{50} inhibiting human cell growth/IC_{50} inhibiting *T. brucei* cell growth) greater than 100.[20] In addition, these compounds showed in vivo activity to decease *T. brucei* cell growth in the infected mice. However, they were not potent enough to clear the infection.[20] Further lead optimization based on the summarized structure activity relationship (SAR) resulted in compound 15′ with better potency and selectivity.[21] Following similar testing it resulted in an IC_{50} of 70 nM to inhibit trypanosomal cell proliferation with a selectivity index around 7000[21]. Unfortunately, this compound has four aromatic moieties (Figure 1), which significantly decreased the solubility and even limited the in vivo testing. The new analogs contain less hydrophobic moieties which should result in a reduced Log P value. To validate this assumption, two methods were employed: a classical slow stir method and computational modeling performed by CHM DRAW.[22] Only three compounds where tested including previous compound 15′ and the current compounds **5** and **57**. Based on the structural similarities it is not necessary to test all the new compounds but the ones with the highest selectivity index. Herein, in this lead optimization, we use different strategies and try to reduce the bulkiness of the compounds.

2. Results and discussion

2.1. Synthesis of the new tubulin inhibitors

In the previous study, the best compound (Figure 1) showed great potency and selectivity to inhibit *T. brucei* cell proliferation. But the congested four aromatic moieties significantly reduce solubility.[21] In order to increase the hydrophilicity, some aromatic moieties should be eliminated. Based on the SAR summarized before, moieties A and B are likely not critical for the anti-trypanosomal activity.[18,20,21] Therefore, in the new design, we used small substituent such as halogens, methyl, and methoxyl groups to occupy the B moiety, and methyl sulfonamide, trifluromethyl sulfonamide and ethyl sulfonamide group to occupy the A moiety as illustrated in Figure 1. For the benzamide moiety, we used different substituents on the aromatic ring to explore the new SAR.

A total of 60 compounds were synthesized using combinatorial chemistry strategy. We modified the R_1, R_2, R_3, R_4 and R_5 moieties of the core structure with different substituents systematically (Figure 1). In these new compounds, the methyl, methoxyl, chloro, and fluoro groups were introduced from the starting materials. Next, the R_2 moiety of the scaffold was modified with different substituted sulfonyl chlorides in order to generate different sulfonamide groups. Then, the nitro group was reduced to amino group in order to introduce the benzamide moiety. The synthesis of these new compounds is illustrated in Schemes 1 and 2.

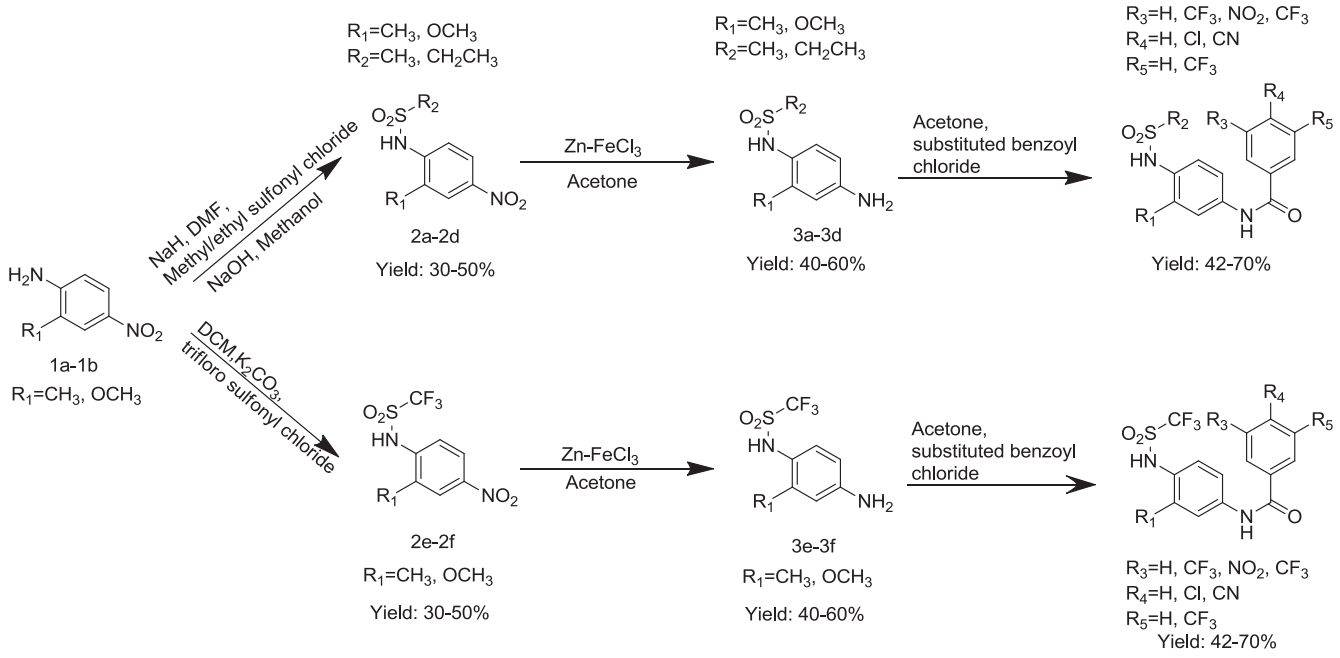
2.2. Biological evaluation of the new derivatives

The biological activity of the synthesized analogs was determined with cell proliferation assays. *T. b. brucei* Lister 427 cells were used as the parasite model. Human normal kidney HEK293 cells and mouse macrophage RAW264.7 cells were used as the mammalian host model.[21] Results of the cell growth inhibition by the compounds are listed in Table 1. The selectivity index is calculated by dividing the IC_{50}s of the mammalian cell growth inhibition with the IC_{50}s of the *T. brucei* cell growth inhibition. Compared to the compounds with IC_{50} below 1 μM in previous study (Figure 1), the new compounds with reduced aromatic moieties showed reduced activity against *T. brucei* cells. However, the study opened up new scaffold for optimization. The compounds with less aromatic moieties are more drug-like and have better potential for the future in vivo study.

For the five moieties (R_1 – R_5) of the compound scaffold (Figure 1), various functional groups were introduced to enhance the anti-trypanosomal activity and decrease the cytotoxicity to mammalian cells. Most compounds have IC_{50} values for mammalian cells above 50 μM, indicting the low toxicity of the compounds. Therefore, the structure activity relationship (SAR) to the mammalian cells of these compounds is not discussed further. For the anti-trypanosomal activity of these compounds, the SAR is summarized in detail. For R_1 domain, there are four different groups (methyl, methoxyl, chloro, and fluoro) in this moiety. Overall, methoxyl group has the lowest activity regardless of the other moieties, and most compounds in this category have IC_{50}s above 10 μM against trypanosomal cell growth. For methyl group as R_1 and R_2, when R_3 and R_5 are trifluoromethyl groups, the resulted compound **5** showed an IC_{50} of 2.93 μM to inhibit trypanosomal cell proliferation, and selectivity index of 56 and 85 to two mammalian cell lines. Whereas R_1 is still methyl, but R_2 is occupied by ethyl or trifluoromethyl groups, activity of these compounds dropped. When R_1 is chloro group and R_2 is methyl group, the compounds lost activity against trypanosomal cells regardless what groups are in R_3-R_5 moiety. When R_2 is changed to ethyl group, the compounds gained some activity, particularly for compound **39** (R_3 = H, R_4 = Cl, R_5 = NO_2) with an IC_{50} of 2.21 μM and compound **40** (R_3 = CF_3, R_4 = H, R_5 = CF_3) with an IC_{50} of 1.73 μM. Unfortunately, the selectivity index of these two compounds are below 50 that is our cutoff to select compounds for further investigation. When R_1 is chloro and R_2 is trifluoromethyl group, the compounds overall are not active as indicated by compounds **41–45**. The combination of fluoro group at R_1 and methyl group at R_2 harms the activity as exhibited by compounds **46–50**. When R_2 is changed to ethyl group such as compounds **54** and **55,** they obtained some activity with IC_{50}s around 8 μM. When R_2 is changed to trifluoromethyl group, the compounds even gained more activity. Particularly for compound **57**, it shows an IC_{50} of 2.1 μM and selectivity index above 119. Considering both the activity and selectivity index, compounds **5** and **57** showed the most promising activity against *T. brucei* cells and also great selectivity with a selectivity index above 50. Further biological investigation was performed with these two compounds.

2.3. Solubility testing for pharmacokinetic profile

The water solubility of compounds **5** and **57** were compared to previously synthesized compound 15′[21] by measuring the LogP

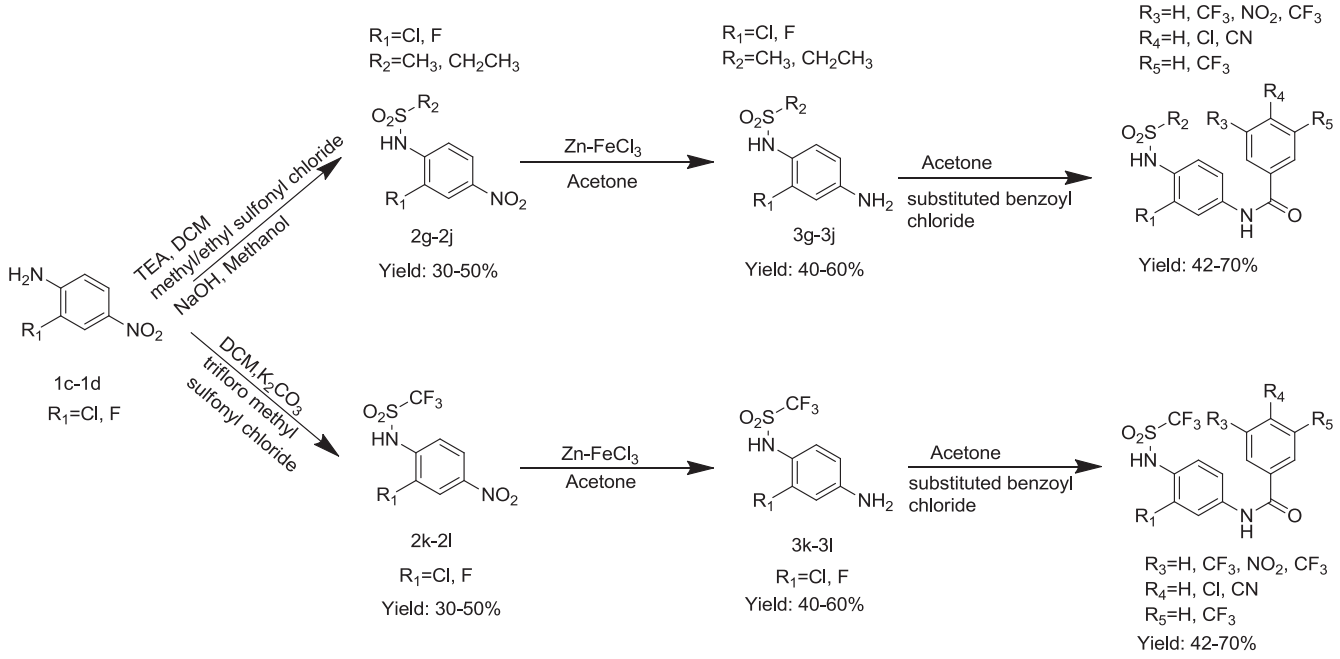


Scheme 1. Synthesis of derivatives (Compounds 1–30).

value.[22,23] In addition, the calculated cLogP values were also obtained with CHEMDRAW. In Table 2, the data are reported and shown that newly synthesized compounds **5** and **57** have a reduced hydrophobicity. It was found that compound **5** has an experimental Log P value of 1.957 ± 0.134 compared with its computational prediction of 3.64 for cLog P. Compound **57** had an experimental log P value of 0.998 ± 0.304 compared with its computational predication cLogP of 3.31. Compound 15′ resulted in an experimental Log P of 2.902 ± 0.199 and the computational prediction cLogP is 5.44. Comparing these values with the predicted values, there are major differences between the predicted and the experimental results. But the hydrophobicity trend of these compounds is consistent from prediction to experiment, and the newly synthesized compounds **5** and **57** have lower log P values, indicating that they are more hydrophilic.

2.4. Biological evaluation of the tubulin targeting effect of compound 5 and compound 57

Our previous study reveals that the lead compound interferes with the microtubule dynamics.[20] However, we removed two aromatic moieties in the new compounds and that is a major structural modification. Although the new analogs still showed activity to selectively inhibit the proliferation of *T. brucei* cells, it is not clear if the tubulin targeting effect still remains after the dramatic structural change, which is necessary to be examined. To test if the new compound is still a tubulin inhibitor, we determined the amount of the polymerized and non-polymerized tubulin after treating *T. brucei* cells with compounds **5** and **57**. We found that the compounds increased the amount of non-polymerized tubulin and decreased the amount of polymerized tubulin in a



Scheme 2. Synthesis of derivatives (Compounds 31–60).

Table 1
Comparison of the growth inhibitory effects of the tubulin inhibitors on mammalian and *T. brucei* cells.

No.	Chemical Structure	IC_{50} value(μM; Mean ± SD)			Selectivity	
		T. brucei	HEK293	RAW264.7	HEK293/*T. brucei*	RAW264.7/*T. brucei*
1	$R_1 = CH_3$, $R_2 = CH_3$ $R_3 = H$, $R_4 = Cl$, $R_5 = H$	> 62.5	50.26 ± 13.57	69.05 ± 20.12	< 1	< 2
2	$R_1 = CH_3$, $R_2 = CH_3$ $R_3 = H$, $R_4 = CN$, $R_5 = H$	> 62.5	43.53 ± 8.54	171.12 ± 68.07	< 1	< 3
3	$R_1 = CH_3$, $R_2 = CH_3$ $R_3 = H$, $R_4 = H$, $R_5 = CF_3$	16.74 ± 10.91	194.3 ± 112.60	109.90 ± 39.79	12	7
4	$R_1 = CH_3$, $R_2 = CH_3$ $R_3 = H$, $R_4 = Cl$, $R_5 = NO_2$	7.57 ± 3.61	73.13 ± 32.60	107.90 ± 50.76	10	14
5	$R_1 = CH_3$, $R_2 = CH_3$ $R_3 = CF_3$, $R_4 = H$, $R_5 = CF_3$	2.93 ± 1.43	163.1 ± 109.62	> 250	56	> 85
6	$R_1 = CH_3$, $R_2 = CH_2CH_3$ $R_3 = H$, $R_4 = Cl$, $R_5 = H$	> 62.5	125.43 ± 27.20	68.71 ± 11.88	< 3	< 2
7	$R_1 = CH_3$, $R_2 = CH_2CH_3$ $R_3 = H$, $R_4 = CN$, $R_5 = H$	> 62.5	> 250	108.76 ± 16.77	ND	< 2
8	$R_1 = CH_3$, $R_2 = CH_2CH_3$ $R_3 = H$, $R_4 = H$, $R_5 = CF_3$	35.26 ± 20.21	209.63 ± 56.26	113.91 ± 28.44	6	3
9	$R_1 = CH_3$, $R_2 = CH_2CH_3$ $R_3 = H$, $R_4 = Cl$, $R_5 = NO_2$	19.42 ± 9.66	144.2 ± 84.16	140.60 ± 80.06	7	7
10	$R_1 = CH_3$, $R_2 = CH_2CH_3$ $R_3 = CF_3$, $R_4 = H$, $R_5 = CF_3$	12.47 ± 9.04	126.1 ± 64.97	124.20 ± 59.80	10	10
11	$R_1 = CH_3$, $R_2 = CF_3$ $R_3 = H$, $R_4 = Cl$, $R_5 = H$	37.21 ± 23.83	200.50 ± 62.06	35.36 ± 14.04	5	1
12	$R_1 = CH_3$, $R_2 = CF_3$ $R_3 = H$, $R_4 = CN$, $R_5 = H$	> 62.5	> 250	135.4 ± 24.58	ND	< 3
13	$R_1 = CH_3$, $R_2 = CF_3$ $R_3 = H$, $R_4 = H$, $R_5 = CF_3$	21.71 ± 14.46	130.60 ± 32.20	8.72 ± 6.18	6	< 1
14	$R_1 = CH_3$, $R_2 = CF_3$ $R_3 = H$, $R_4 = Cl$, $R_5 = NO_2$	> 62.5	> 250	89.78 ± 24.65	ND	< 2
15	$R_1 = CH_3$, $R_2 = CF_3$ $R_3 = CF_3$, $R_4 = H$, $R_5 = CF_3$	20.65 ± 8.02	181.65 ± 89.65	40.66 ± 15.77	9	2
16	$R_1 = OCH_3$, $R_2 = CH_3$ $R_3 = H$, $R_4 = Cl$, $R_5 = H$	> 62.5	> 250	70.00 ± 16.32	ND	< 2
17	$R_1 = OCH_3$, $R_2 = CH_3$ $R_3 = H$, $R_4 = CN$, $R_5 = H$	> 62.5	> 250	> 250	ND	ND
18	$R_1 = OCH_3$, $R_2 = CH_3$ $R_3 = H$, $R_4 = H$, $R_5 = CF_3$	> 62.5	> 250	84.77 ± 27.70	ND	< 2
19	$R_1 = OCH_3$, $R_2 = CH_3$ $R_3 = H$, $R_4 = Cl$, $R_5 = NO_2$	> 62.5	164.41 ± 37.51	> 250	< 3	ND
20	$R_1 = OCH_3$, $R_2 = CH_3$ $R_3 = CF_3$, $R_4 = H$, $R_5 = CF_3$	> 62.5	41.97 ± 17.57	> 250	< 1	ND
21	$R_1 = OCH_3$, $R_2 = CH_2CH_3$ $R_3 = H$, $R_4 = Cl$, $R_5 = H$	> 62.5	> 250	60.09 ± 31.85	ND	< 1
22	$R_1 = OCH_3$, $R_2 = CH_2CH_3$ $R_3 = H$, $R_4 = CN$, $R_5 = H$	> 62.5	> 250	66.44 ± 29.82	ND	< 2
23	$R_1 = OCH_3$, $R_2 = CH_2CH_3$ $R_3 = H$, $R_4 = H$, $R_5 = CF_3$	35.74 ± 24.93	> 250	84.83 ± 41.66	> 7	2
24	$R_1 = OCH_3$, $R_2 = CH_2CH_3$ $R_3 = H$, $R_4 = Cl$, $R_5 = NO_2$	> 62.5	63.65 ± 18.13	5.66 ± 2.95	< 2	< 1
25	$R_1 = OCH_3$, $R_2 = CH_2CH_3$ $R_3 = CF_3$, $R_4 = H$, $R_5 = CF_3$	11.55 ± 6.21	> 250	> 250	> 22	> 22
26	$R_1 = OCH_3$, $R_2 = CF_3$ $R_3 = H$, $R_4 = Cl$, $R_5 = H$	45.55 ± 24.55	> 250	108.48 ± 46.66	> 5	2
27	$R_1 = OCH_3$, $R_2 = CF_3$ $R_3 = H$, $R_4 = CN$, $R_5 = H$	9.48 ± 3.91	> 250	> 250	> 26	> 26
28	$R_1 = OCH_3$, $R_2 = CF_3$ $R_3 = H$, $R_4 = H$, $R_5 = CF_3$	43.04 ± 23.44	> 250	75.23 ± 14.75	> 6	2
29	$R_1 = OCH_3$, $R_2 = CF_3$ $R_3 = H$, $R_4 = Cl$, $R_5 = NO_2$	14.08 ± 6.89	> 250	> 250	> 18	> 18
30	$R_1 = OCH_3$, $R_2 = CF_3$ $R_3 = CF_3$, $R_4 = H$, $R_5 = CF_3$	> 62.5	> 250	> 250	ND	ND
31	$R_1 = Cl$, $R_2 = CH_3$ $R_3 = H$, $R_4 = Cl$, $R_5 = H$	> 62.5	167.45 ± 30.03	79.94 ± 49.27	< 3	< 2
32	$R_1 = Cl$, $R_2 = CH_3$ $R_3 = H$, $R_4 = CN$, $R_5 = H$	> 62.5	> 250	> 250	ND	ND
33	$R_1 = Cl$, $R_2 = CH_3$ $R_3 = H$, $R_4 = H$, $R_5 = CF_3$	> 62.5	> 250	> 250	ND	ND
34	$R_1 = Cl$, $R_2 = CH_3$ $R_3 = H$, $R_4 = Cl$, $R_5 = NO_2$	> 62.5	203.57 ± 29.44	43.60 ± 22.23	< 4	< 1
35	$R_1 = Cl$, $R_2 = CH_3$ $R_3 = CF_3$, $R_4 = H$, $R_5 = CF_3$	10.08 ± 6.58	184.56 ± 84.83	69.55 ± 29.89	18	7
36	$R_1 = Cl$, $R_2 = CH_2CH_3$ $R_3 = H$, $R_4 = Cl$, $R_5 = H$	19.12 ± 13.52	> 250	> 250	> 13	> 13
37	$R_1 = Cl$, $R_2 = CH_2CH_3$ $R_3 = H$, $R_4 = CN$, $R_5 = H$	37.95 ± 20.73	> 250	226.08 ± 114.5	> 7	6
38	$R_1 = Cl$, $R_2 = CH_2CH_3$ $R_3 = H$, $R_4 = H$, $R_5 = CF_3$	7.81 ± 4.93	158.54 ± 72.35	62.43 ± 19.90	20	8
39	$R_1 = Cl$, $R_2 = CH_2CH_3$ $R_3 = H$, $R_4 = Cl$, $R_5 = NO_2$	2.21 ± 1.08	63.80 ± 32.72	125.40 ± 62.97	29	57
40	$R_1 = Cl$, $R_2 = CH_2CH_3$ $R_3 = CF_3$, $R_4 = H$, $R_5 = CF_3$	1.73 ± 0.71	32.17 ± 14.30	64.07 ± 53.30	19	37
41	$R_1 = Cl$, $R_2 = CF_3$ $R_3 = H$, $R_4 = Cl$, $R_5 = H$	> 62.5	190.90 ± 63.12	47.15 ± 6.39	< 4	< 1
42	$R_1 = Cl$, $R_2 = CF_3$ $R_3 = H$, $R_4 = CN$, $R_5 = H$	11.00 ± 8.06	> 250	> 250	> 23	> 23
43	$R_1 = Cl$, $R_2 = CF_3$ $R_3 = H$, $R_4 = H$, $R_5 = CF_3$	> 62.5	209.85 ± 39.02	55.82 ± 7.79	< 4	< 1
44	$R_1 = Cl$, $R_2 = CF_3$ $R_3 = H$, $R_4 = Cl$, $R_5 = NO_2$	47.63 ± 26.11	228.48 ± 44.82	31.68 ± 7.26	5	1
45	$R_1 = Cl$, $R_2 = CF_3$ $R_3 = CF_3$, $R_4 = H$, $R_5 = CF_3$	47.36 ± 27.31	> 250	136.06 ± 51.81	> 5	3
46	$R_1 = F$, $R_2 = CH_3$ $R_3 = H$, $R_4 = Cl$, $R_5 = H$	> 62.5	216.21 ± 99.88	22.25 ± 6.64	< 4	< 1
47	$R_1 = F$, $R_2 = CH_3$ $R_3 = H$, $R_4 = CN$, $R_5 = H$	> 62.5	> 250	> 250	ND	ND
48	$R_1 = F$, $R_2 = CH_3$ $R_3 = H$, $R_4 = H$, $R_5 = CF_3$	> 62.5	> 250	32.29 ± 15.69	ND	< 1
49	$R_1 = F$, $R_2 = CH_3$ $R_3 = H$, $R_4 = Cl$, $R_5 = NO_2$	> 62.5	23.08 ± 3.13	101.79 ± 45.41	< 1	< 2
50	$R_1 = F$, $R_2 = CH_3$ $R_3 = CF_3$, $R_4 = H$, $R_5 = CF_3$	23.68 ± 16.82	> 250	124.94 ± 70.28	> 11	5
51	$R_1 = F$, $R_2 = CH_2CH_3$ $R_3 = H$, $R_4 = Cl$, $R_5 = H$	> 62.5	53.37 ± 4.83	151.92 ± 73.77	< 1	< 3
52	$R_1 = F$, $R_2 = CH_2CH_3$ $R_3 = H$, $R_4 = CN$, $R_5 = H$	> 62.5	26.36 ± 6.79	107.38 ± 49.13	< 1	< 2
53	$R_1 = F$, $R_2 = CH_2CH_3$ $R_3 = H$, $R_4 = H$, $R_5 = CF_3$	33.91 ± 19.71	94.84 ± 29.38	119.17 ± 43.96	3	4
54	$R_1 = F$, $R_2 = CH_2CH_3$ $R_3 = H$, $R_4 = Cl$, $R_5 = NO_2$	8.39 ± 5.68	83.66 ± 41.60	198.43 ± 58.95	10	24
55	$R_1 = F$, $R_2 = CH_2CH_3$ $R_3 = CF_3$, $R_4 = H$, $R_5 = CF_3$	8.78 ± 5.87	> 250	> 250	> 28	> 28
56	$R_1 = F$, $R_2 = CF_3$ $R_3 = H$, $R_4 = Cl$, $R_5 = H$	> 62.5	78.98 ± 41.12	3.27 ± 1.78	< 2	< 1
57	$R_1 = F$, $R_2 = CF_3$ $R_3 = H$, $R_4 = CN$, $R_5 = H$	2.10 ± 1.01	> 250	> 250	> 119	> 119
58	$R_1 = F$, $R_2 = CF_3$ $R_3 = H$, $R_4 = H$, $R_5 = CF_3$	47.35 ± 29.86	151.72 ± 31.83	34.44 ± 20.47	3	1
59	$R_1 = F$, $R_2 = CF_3$ $R_3 = H$, $R_4 = Cl$, $R_5 = NO_2$	16.79 ± 12.71	193.67 ± 97.12	187.75 ± 93.75	12	11
60	$R_1 = F$, $R_2 = CF_3$ $R_3 = CF_3$, $R_4 = H$, $R_5 = CF_3$	9.386 ± 5.49	163.76 ± 90.18	112.41 ± 55.98	17	12

IC_{50} values represent the Mean ± Standard Deviation (S.D.) of four parallel measurements.
"ND" = not determined.

dose-dependent manner (Figure 2), indicating that after the treatment, the tubulin polymerization in the cells was inhibited by the compounds. Compound **5** showed much better activity than compound **57** to inhibit tubulin polymerization. However, the IC_{50}s against trypanosomal cell proliferation of the two compounds are similar. It suggests that compound **57** may also target the cell proliferation via other mechanisms, which compensates the relative lower tubulin targeting effect in comparison to compound **5**. Overall, the results demonstrate that the new

Table 2
Lop P values assay of compound 5, compound 57 and compound 15′

	Compound 5 (λ = 290 nm)	Compound 57 (λ = 241 nm)	Compound 15′ (λ = 212 nm)
experimental Log P	1.957 ± 0.134	0.998 ± 0.304	2.902 ± 0.199
Predicted cLogP	3.64	3.31	5.44

The measurement was repeated three times with water and 1-octanol as the two solvents, and the results are presented as Mean ± SD, cLogP is determined using ChemDraw Ultra 12.0.2.1076.

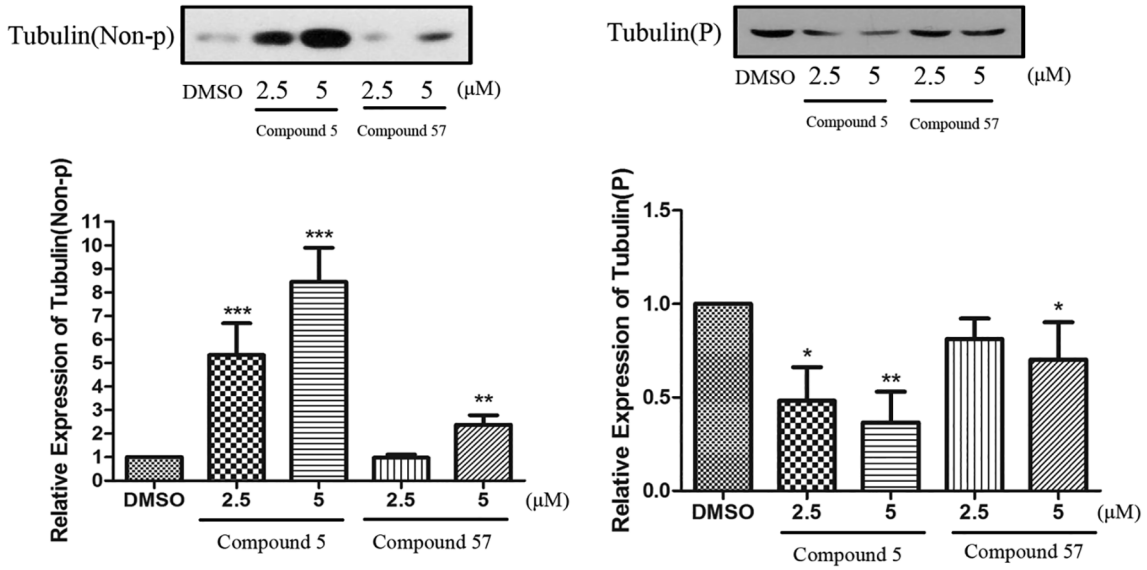


Figure 2. Tubulin inhibitor compound **5** and compound **57** inhibit the tubulin polymerization in *T. brucei* cells. After treatment, the level of polymerized tubulin is significantly decreased. The band intensities of HEXIM1 and β-actin were quantified using ImageJ (NIH) to generate the figure. The figures are representative of 3 experiments. *$p < 0.05$ vs. DMSO, **$p < 0.01$ vs DMSO, *** $p < 0.001$ by unpaired *t*-test.

compounds are still tubulin inhibitors and selectively target the tubulin dynamic regulation in *T. brucei* cells. They are potent and selective tubulin inhibitors against trypanosomiasis, and could be the new lead for further optimization.

3. Conclusion

Due to limitations of current anti-trypanosomiasis drugs, the development of new medication for the treatment of African trypanosomiasis is still urgently needed. In the present study we focused on the development of selective tubulin inhibitors for the treatment of this disease. Based on the inhibitory effects of the compounds on *T. brucei* cell proliferation and mammalian cell growth, the selectivity index is determined in order to identify more potent and selective drug candidates. This resulted in compounds with an IC_{50}s around 2 μM to inhibit the growth of *T. brucei* cells, and not affecting the viability of mammalian cells even with a concentration that is a hundred folds higher. Compared to previous studies, the new compounds have only two aromatic moieties, which increased the solubility and ligand efficacy. Furthermore, the selective tubulin inhibitor interferes with tubulin polymerization in *T. brucei* cells and significantly increased the non-polymerized tubulin and decreased the polymerized tubulin.

4. Experimental section

4.1. Chemistry

Chemicals were commercially available and used as received without further purification unless otherwise noted. Moisture sensitive reactions were carried out under a dry argon atmosphere in flame-dried glassware. Thin layer chromatography was performed on silica gel TLC plates with a fluorescence indicator at 254 nm (Fluka). Flash column chromatography was performed using silica gel 60 Å (BDH, 40–63 μm). Mass spectra were obtained on a Bruker Ion-Trap Mass Spectrometer at Cleveland State University MS facility Center. All the NMR spectra were recorded on a Bruker 400 MHz spectrometer using $CDCl_3$ or DMSO-d_6 as the solvent.

Reversed-phase HPLC analysis of compounds was conducted on a Beckman HPLC system with an Auto Sampler. The chromatographic separation was performed on a C_{18} column (2.0 mm × 150 mm, 5 μm) from Phenomenex (Torrance, CA). The mobile phase was employed for isocratic elution with a flow rate of 0.2 mL/min. The injection volume was 20 μL and the UV detector was set up at 260 nm.

The synthesis procedures of compounds **1–60** are listed below.

4.1.1. General method for the preparation of 2a-2d

Sulfonamides **2a-2d** were prepared from arylsubstituted 2-amino-5-nitrophenols **1a-1b**. Dissolve arylsubstituted 2-amino-5-nitrophenol (15.0 mM) in 40 mL anhydrous DMF add NaH (95% powder, 1.325 g, 52.5 mM, 3.5 equiv) into the solution stir at room temperature for 30 min. After stir at room temperature for 30 min corresponding sulfonyl chloride (45 mM, 3 equiv) was added to the mixture the reaction continued overnight at room temperature. Quench the reaction with water and the mixture was neutralized with 6 N HCl until pH = 1–2, yellow intermediate precipitated. Intermediate was collected by filtration and wash with water, which was used to the next reaction without further purification

The intermediate was dissolved in 100 mL methanol and 50 mL 4 N NaOH aq solution was added into the solution, stir at room temperature for 2 h. After reaction completed neutralized the solution with 6 N HCl until pH = 1–2. The precipitate was collected by filtration and was with water and cold ether to provide desire product.

4.1.2. General method for the preparation of 2g-2j

Sulfonamides **2g-2j** were prepared from aryl-substituted 2-amino-5-nitrophenols **1c-1d**. Dissolve arylsubstituted 2-amino-5-nitrophenol (15.0 mM) in 150 mL anhydrous DCM, TEA was added into solution (105.0 mM, 7 equiv). After TEA added corresponding sulfonyl chloride (45 mM, 3 equiv) added into solution, reacted at room temperature overnight. After reaction completed DCM evaporated under vacuum added 200 mL water added into the flask neutralized with 6 N HCl until pH = 1–2. Collect the solid intermediate by filtration. Wash the intermediate with water, which was used to the next reaction without further purification.

The intermediate was dissolved in 100 mL methanol and 50 mL 4 N NaOH aq solution was added into the solution, stir at room temperature for 2 h. After reaction completed neutralized the solution with 6 N HCl until pH = 1–2. The precipitate was collected by filtration and was with water and cold ether to provide desire product

4.1.3. General method for the preparation of 2e-2f and 2k-2 l

Trifluoroumethylsulfonamides **2e-2f** and **2k-2l** were prepared from aryl-substituted 2-amino-5-nitrophenols **1a-1d**. Dissolve arylsubstituted 2-amino-5-nitrophenol (15.0 mM) in 150 mL anhydrous DCM and K_2CO_3 (75 mM) was added to solution, then cool to 0 °C. Trifluoromethanesulfonic anhydrous (45 mM) was added into the solution dropwise. The resulting mixture was continuously stirred for 3 h at 0–5 °C. Water (30 mL) was added to quench the reaction. DCM was evaporated under vacuum, and then 6 N HCl (10 mL) was added to acidify the residue. The product was collected by filtration and washed with water and cool ether, then used for the next reaction without further purification.

4.1.4. General method for the preparation of product 1–60

Dissolve intermediates **2a-2 l** 1 mM in acetone 10 mL and water 1 mL. After intermediates dissolve into solvent Zn (10 mM, 10 equiv) and $FeCl_3$ (4 mM, 4 equiv) were added into solution, the result mixture was stirred at room temperature for 1 h. After the reaction completed the mixture was filtrate through celite to remove inorganic precipitate. The eluent was evaporated under vacuum(**3a-3 l**).

Redissolve the intermediate into 10 mL acetone, the corresponding benzoyl chloride (1.1 mM, 1.1 equiv) was added then 10 mL brine and 10 mL saturated Na_2CO_3 solution. The result mixture was stirred at room temperature for 1 h. After the reaction completed neutralized with 6 N HCl until pH = 1–2, acetone was evaporated under vacuum. The product was collected by filtration and purify by recrystallization in ethanol/ water (3:1).

4.1.5. 4-Chloro-N-(4-(Methylsulfonamido)-3-Methylphenyl)Benzamide (1).

Yield 56.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.43 (s, 1H), 8.36 (s, 1H), 8.01 (d, 2H, J = 8.0), 7.75 (s, 1H), 7.74 (d, 1H, J = 8.0), 7.63 (d, 2H, J = 8.0), 7.33 (d, 1H, J = 8.0), 3.25 (s, 3H), 2.34 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.95, 156.41, 138.95, 137.07, 133.81, 133.46, 130.12 (2C), 128.99 (2C), 128.95, 125.69, 123.00, 119.31, 43.62, 37.37, 18.17(solvent). DUIS-MS calculated for $C_{15}H_{15}ClN_2O_3S$, $[M-H]^-$: *m/z* 337.04, found *m/z* 336.9. Purity: 95.3%.

4.1.6. 4-Cyano-N-(4-(Methylsulfonamido)-3-Methylphenyl)Benzamide (2).

Yield 60.5%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.61 (s, 1H), 8.36 (s, 1H), 8.14 (d, 2H, J = 8.4), 8.05 (d, 2H, J = 8.4), 7.76 (s, 1H), 7.74 (d, 1H, J = 8.0), 7.34 (d, 1H, J = 8.0), 3.25 (s, 3H), 2.35 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.66, 139.13, 133.49, 132.98 (2C), 132.95, 129.03 (2C), 128.96, 125.62, 123.07, 119.39, 118.75, 114.48, 43.59, 18.19. DUIS-MS calculated for $C_{16}H_{15}N_3O_3S$, $[M-H]^-$: *m/z* 328.08, found *m/z* 328.0. Purity: 97.3%.

4.1.7. N-(4-(Methylsulfonamido)-3-Methylphenyl)-3-(Trifluoromethyl) Benzamide (3).

Yield 62.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.45 (s, 1H), 9.01 (s, 1H), 8.30 (s, 1H), 8.27 (d, 1H, J = 8.0), 7.98 (d, 1H, J = 8.0) 7.80 (t, 1H, J = 8.0), 7.68 (s, 1H), 7.63 (d, 1H, J = 8.8), 7.28 (d, 1H, J = 8.8), 2.98 (s, 3H), 2.33 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.39, 137.52, 136.18, 135.47, 132.29, 131.71, 130.22, 130.16, 129.84, 129.52, 129.21 (q, 1C), 128.64 (m, 1C), 127.52, 124.73, 124.69, 124.66, 124.62 (q, 1C), 123.14, 119.16, 31.14, 18.84. DUIS-MS calculated for $C_{16}H_{15}F_3N_2O_3S$, $[M-H]^-$: *m/z* 371.07, found *m/z* 370.9. Purity: 97.8%.

4.1.8. 4-Chloro-N-(4-(Methylsulfonamido)-3-Methylphenyl)-3-Nitrobenzamide (4).

Yield 61.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.73 (s, 1H), 8.69 (s, 1H), 8.38 (s, 1H), 8.32 (d, 1H, J = 8.4), 7.79 (d, 1H, J = 8.4), 7.76 (s, 1H), 7.75 (d, 1H, J = 8.8), 7.35 (d, 1H, J = 8.8), 3.27 (s, 3H), 2.36 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.98, 156.41, 147.96, 138.50, 135.08, 133.60, 133.37, 132.47, 128.69, 125.77, 125.37, 123.15, 119.44, 56.50(solvent) 43.61, 37.52, 18.23(solvent). DUIS-MS calculated for $C_{15}H_{14}ClN_3O_2S$, $[M-H]^-$: *m/z* 382.03, found *m/z* 381.9. Purity: 98.2%.

4.1.9. N-(4-(Methylsulfonamido)-3-Methylphenyl)-3,5-Bis (Trifluoromethyl)Benzamide (5).

Yield 55.4%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.59 (s, 1H), 9.57 (s, 1H), 8.62 (s, 2H), 8.38 (s, 1H), 7.64 (s, 1H), 7.74 (d, 1H, J = 8.6), 7.58 (d, 1H, J = 8.6), 3.33 (s, 3H) ,2.26 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.70(solvent), 160.21, 137.56, 135.46, 132.52, 131.14, 130.81, 130.43 (q, 1C), 128.97 (m, 1C), 125.56 (m, 1C), 124.97, 123.78, 123.08, 122.26, 119.56, 119.02, 18.51(solvent). DUIS-MS calculated for $C_{17}H_{14}F_6N_2O_3S$, $[M-H]^-$: *m/z* 439.06, found *m/z* 439.0. Purity: 98.8%.

4.1.10. 4-Chloro-N-(4-(Ethylsulfonamido)-3-Methylphenyl)Benzamide (6).

Yield 68.9%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.30 (s, 1H), 8.99 (s, 1H), 7.99 (d, 2H, J = 8.8), 7.66 (d, 1H, J = 2.4), 7.62 (d, 2H, J = 8.8), 7.58 (dd, 1H, J_1 = 2.4, J_2 = 8.8), 7.23 (d, 1H, J = 8.8), 3.08 (q, 2H, J = 8.0), 2.32 (s, 3H), 1.28 (t, 3H, J = 8.0). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.80, 137.55, 136.90, 135.29, 134.01, 131.54, 130.06 (2C), 128.94 (2C), 127.30, 122.99, 119.04, 46.67, 18.92, 8.56. DUIS-MS calculated for $C_{16}H_{17}ClN_2O_3S$, $[M-H]^-$: *m/z* 351.06, found *m/z* 351.0; Purity: 96.7%.

4.1.11. 4-Cyano-N-(4-(Ethylsulfonamido)-3-Methylphenyl)Benzamide (7).

Yield 60.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.45 (s, 1H), 9.00 (s, 1H), 8.11 (d, 2H, J = 8.4), 8.04 (d, 2H, J = 8.4), 7.67 (d, 1H, J = 2.4), 7.59 (dd, 1H, J_1 = 2.4, J_2 = 8.4,), 7.25 (d, 1H, J = 8.4), 3.08 (q, 2H, J = 7.6), 2.33 (s, 3H), 1.27 (t, 3H, J = 7.6). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.49, 139.34, 137.28, 135.29, 132.95, 131.82, 128.87, 127.27, 123.03, 119.09, 118.78, 114.34, 46.70, 18.91, 8.56. DUIS-MS calculated for $C_{17}H_{17}N_3O_3S$, $[M-H]^-$: *m/z* 342.09, found *m/z* 341.8. Purity: 96.8%.

4.1.12. N-(4-(Ethylsulfonamido)-3-Methylphenyl)-3-(Trifluoromethyl) Benzamide (8).

Yield 61.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.43 (s, 1H), 8.99 (s, 1H), 8.30 (s, 1H), 8.27 (d, 1H, J = 7.6), 7.98 (d, 1H, J = 7.6), 7.80 (t, 1H, J = 7.6), 7.67 (s, 1H), 7.61 (d, 1H, J = 8.4), 7.26 (d, 1H, J = 8.4), 3.09 (q, 2H, J = 7.2), 2.34 (s, 3H), 1.28 (t, 3H, J = 7.2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.37, 137.35, 136.19, 135.29, 132.29, 131.76, 130.21, 130.16, 129.84, 129.52, 129.18 (q, 1C), 128.63 (m, 1C), 127.27, 124.69 (m, 1C), 123.14, 119.17, 46.73, 19.00, 8.56. DUIS-MS calculated for $C_{17}H_{17}F_3N_2O_3S$, $[M-H]^-$: *m/z* 385.08, found *m/z* 384.8. Purity: 97.5%.

4.1.13. 4-Chloro-N-(4-(Ethylsulfonamido)-3-Methylphenyl)-3-Nitrobenzamide (9).

Yield 64.3%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.52 (s, 1H), 9.02 (s, 1H), 8.63 (d, 1H, J = 2.0), 8.26 (dd, 1H, J_1 = 8.4, J_2 = 2.0), 7.98 (d, 1H, J = 8.4), 7.65 (d, 1H, J = 2.0), 7.59 (dd, 1H, J_1 = 2.0, J_2 = 8.6), 7.26 (d, 1H, J = 8.6), 3.08 (q, 2H, J = 7.3), 2.33 (s, 3H), 1.27 (t, 3H, J = 7.3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.91, 137.06, 135.32, 133.29 (2C), 132.45, 131.96, 128.56, 127.28, 125.27 (2C), 123.09, 119.14, 46.74, 18.89, 8.55. DUIS-MS calculated for $C_{16}H_{16}ClN_3O_5S$, $[M-H]^-$: *m/z* 396.04, found *m/z* 396.0. Purity: 98.8%.

4.1.14. N-(4-(Ethylsulfonamido)-3-Methylphenyl)-3,5-Bis (Trifluoromethyl)Benzamide (10).

Yield 54.6%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.63 (s, 1H), 9.02 (s, 1H), 8.62 (s, 2H), 8.38 (s, 1H), 7.66 (s, 1H), 7.63 (d, 1H, J = 8.4), 7.29 (d, 1H, J = 8.4), 3.08 (q, 2H, J = 7.2), 2.35 (s, 3H), 1.29, (t, 3H, J = 7.2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.95, 137.51, 136.98, 135.29, 132.09, 131.49, 131.16, 130.82, 130.49 (q, 1C), 129.02 (m, 2C), 127.24, 125.56 (m, 1C), 123.26, 119.29, 46.79, 18.88, 8.56. DUIS-MS calculated for $C_{18}H_{16}F_6N_2O_3S$, $[M-H]^-$: *m/z* 453.07, found *m/z* 453.0. Purity: 98.8%.

4.1.15. 4-Chloro-N-(3-Methyl-4-Trifluoromethanesulfonylamino-Phenyl) Benzamide (11).

Yield 59.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.34 (s, 1H), 10.37 (s, 1H), 7.99 (d, 2H, J = 8.4), 7.74 (s, 1H), 7.64 (m, 3H), 7.30 (d, 1H, J = 8.8), 2.32 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.98, 139.21, 137.02, 136.34, 133.89, 130.10 (2C), 128.96 (2C), 128.91, 128.38, 122.90, 121.80, 119.19. DUIS-MS calculated for $C_{15}H_{12}ClF_3N_2O_3S$, $[M-H]^-$: *m/z* 391.01, found *m/z* 390.9. Purity: 95.7%.

4.1.16. 4-Cyano-N-(3-Methyl-4-Trifluoromethanesulfonylamino-Phenyl) Benzamide (12).

Yield 52.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.40 (s, 1H), 10.54 (s, 1H), 8.11 (d, 2H, J = 8.0), 8.04 (d, 2H, J = 8.0), 7.75 (s, 1H), 7.65 (d, 2H, J = 8.8), 7.25 (d, 1H, J = 8.8), 2.33 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.720, 139.211, 138.969, 136.400, 132.867 (2C), 129.013 (2C), 128.965, 128.636, 122.942, 119.426, 118.75, 114.45, 18.34. DUIS-MS calculated for $C_{16}H_{12}F_3N_3O_3S$, $[M-H]^-$: *m/z* 382.05, found *m/z* 381.9. Purity: 97.9%.

4.1.17. N-(3-Methyl-4-Trifluoromethanesulfonylamino-Phenyl)-3-Trifluoromethyl-Benzamide (13).

Yield 55.4%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.55 (s, 1H), 8.27 (s, 1H), 8.25 (d, 1H, J = 8.0), 7.97 (d, 1H, J = 8.0), 7.79 (t, 1H, J = 8.0), 7.72 (s, 1H), 7.66 (d, 1H, J = 8.4), 7.24 (d, 1H, J = 8.4), 2.32 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.72, 139.00, 136.50, 135.98, 132.28, 130.29, 130.20, 129.88, 129.56, 129.24 (q, 1C), 129.00, 128.77 (m, 1C), 128.51, 124.70 (m, 1C), 123.11, 119.37, 18.29. DUIS-MS calculated for $C_{16}H_{12}F_6N_2O_3S$, $[M-H]^-$: *m/z* 425.04, found *m/z* 424.8. Purity: 98.7%.

4.1.18. 4-Chloro-N-(3-Methyl-4-Trifluoromethanesulfonylamino-Phenyl)-3-Mitro-Benzamide (14).

Yield 52.9%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.38 (s, 1H), 10.63 (s, 1H), 8.65 (s, 1H), 8.27 (d, 1H, J = 8.4), 7.99 (d, 1H, J = 8.4), 7.74 (s, 1H), 7.66 (d, 1H, J = 8.6), 7.26 (d, 1H, J = 8.6), 2.33 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.03, 147.89, 138.81, 136.46, 135.15, 133.34, 132.47, 129.01, 128.72, 128.67, 125.34, 123.01, 119.30, 18.34. DUIS-MS calculated for $C_{15}H_{11}ClF_3N_3O_5S$, $[M-H]^-$: *m/z* 436.00, found *m/z* 435.9. Purity: 96.8%.

4.1.19. N-(3-Methyl-4-Trifluoromethanesulfonylamino-Phenyl)-3,5-Bis-Trifluoromethyl-Benzamide (15).

Yield 53.6%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.69 (s, 1H), 8.61 (s, 2H), 8.46 (s, 1H), 8.39 (s, 1H), 7.72 (s, 1H), 7.67 (d, 1H, J = 8.8), 7.28 (d, 1H, J = 8.8), 2.34 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.07, 138.65, 137.41, 136.42, 131.50, 131.17, 130.84, 130.51 (q, 2C), 129.02 (m, 2C), 128.97, 125.72 (m, 1C), 127.66, 124.95, 122.23, 119.52 (q, 2CF_3), 123.16, 121.90, 119.41, 18.32. DUIS-MS calculated for $C_{17}H_{11}F_9N_2O_3S$, $[M-H]^-$: *m/z* 493.03, found *m/z* 492.8, Purity: 98.1%.

4.1.20. 4-Chloro-N-(4-(Methylsulfonamido)-3-Methoxylphenyl) Benzamide (16).

Yield 69.8%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.34 (s, 1H), 8.84 (s, 1H), 8.00 (d, 2H, J = 8.4), 7.63 (d, 2H, J = 8.4), 7.62 (s, 1H), 7.35 (d, 1H, J = 8.8), 7.22 (d, 1H, J = 8.8), 3.84 (s, 3H), 2.93 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.83, 153.56, 139.44, 136.60, 134.00, 130.06 (2C), 129.92 (2C), 127.37, 121.53, 112.76, 104.90, 56.15, 40.06. DUIS-MS calculated for $C_{15}H_{15}ClN_2O_4S$, $[M-H]^-$: *m/z* 353.04, found *m/z* 352.9. Purity: 96.7%.

4.1.21. 4-Cyano-N-(4-(Methylsulfonamido)-3-Methoxylphenyl)Benzamide (17).

Yield 65.3%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.51 (s, 1H), 8.87 (s, 1H), 8.12 (d, 2H, J = 8.4), 8.05 (d, 2H, J = 8.4), 7.62 (d, 1H, J = 2.0), 7.34 (dd, 1H, J_1 = 2.0, J_2 = 8.8), 7.23 (d, 1H, J = 8.8), 3.84 (s, 3H), 2.93 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ:164.56, 153.53, 139.31, 138.29, 132.96, 128.96, 127.33, 121.99, 118.77, 114.40, 112.81, 104.91, 56.16, 40.07. DUIS-MS calculated for $C_{16}H_{15}N_3O_4S$, $[M-H]^-$: *m/z* 344.07, found *m/z* 344.0. Purity: 98.0%.

4.1.22. N-(4-(Methylsulfonamido)-3-Methoxylphenyl)-3-(Trifluoromethyl)Benzamide (18).

Yield 65.3%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.50 (s, 1H), 8.86 (s, 1H), 8.30 (s, 1H), 8.28 (d, 1H, J = 7.6), 7.99 (d, 1H, J = 7.6), 7.81 (t, 1H, J = 7.6), 7.62 (s, 1H), 7.37 (d, 1H, J = 8.8), 7.24 (d, 1H, J = 8.8), 3.85 (s, 3H), 2.94 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.46, 153.54, 138.35, 136.18, 132.28, 130.23, 130.18, 129.86, 129.54, 129.22 (q, 1C), 128.66 (m, 1C), 127.29, 124.69 (m, 1C), 121.96, 112.92, 105.05, 56.19, 40.07. DUIS-MS calculated for $C_{16}H_{15}F_3N_2O_4S$, $[M-H]^-$: 387.06, found 386.9. Purity: 97.0%.

4.1.23. 4-Chloro-N-(4-(Methylsulfonamido)-3-Methoxylphenyl)-3-Nitrobenzamide (19).

Yield 70.1%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.57 (s, 1H), 8.88 (s, 1H), 8.65 (s, 1H), 8.28 (d, 1H, J = 8.4), 7.99 (d, 1H, J = 8.4), 7.59 (s, 1H), 7.36 (d, 1H, J = 8.4), 7.25 (d, 1H, J = 8.4), 3.84 (s, 3H), 2.94 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.97, 153.50, 147.97, 138.04, 135.27, 133.28, 132.47, 128.60, 127.26, 125.26, 122.20, 112.92, 106.01, 56.20, 40.06. DUIS-MS calculated for $C_{15}H_{14}ClN_3O_6S$, $[M-H]^-$: *m/z* 398.02, found *m/z* 397.8. Purity: 98.3%.

4.1.24. N-(4-(Methylsulfonamido)-3-Methoxylphenyl)-3,5-Bis (trifluoromethyl)Benzamide (20).

Yield 63.4%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.69 (s, 1H), 8.90 (s, 1H), 8.62 (s, 2H), 8.39 (s, 1H), 7.59 (d, 1H, J = 2.0), 7.37 (dd, 1H, J_1 = 2.0, J_2 = 8.8), 7.27 (d, 1H, J = 8.8), 3.85 (s, 3H), 2.95 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.95, 153.48, 137.92, 137.49, 131.49, 131.16, 130.83, 130.50 (q, 2C), 129.02 (m, 2C), 127.21, 125.63 (m, 1C), 127.68, 124.96, 122.25, 199.53 (q, 2CF_3), 122.31, 113.08, 105.15, 56.21, 40.04. DUIS-MS calculated for $C_{17}H_{14}F_6N_2O_4S$, $[M-H]^-$: *m/z* 455.05, found *m/z* 454.9. Purity: 96.1%.

4.1.25. 4-Chloro-N-(4-(Ethylsulfonamido)-3-Methoxylphenyl)Benzamide (21).

Yield 69.9%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.35 (s, 1H), 8.84 (s, 1H), 7.99 (d, 2H, J = 8.4), 7.63 (d, 2H, J = 8.4), 7.61 (d, 1H, J = 2.0), 7.34 (dd, 1H, J_1 = 2.0, J_2 = 8.4) ,7.23 (d, 1H, J = 8.4), 3.82 (s, 3H), 2.99 (q, 2H, J = 7.2), 1.26 (t, 3H, J = 7.2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.93, 153.38, 138.44, 136.95, 134.00, 130.06, 128.96,

127.25, 121.73, 112.74, 104.80, 56.11, 46.61, 8.48. DUIS-MS calculated for $C_{16}H_{17}ClN_2O_4S$, $[M-H]^-$: *m/z* 367.05, found *m/z* 366.9, Purity: 97.5%.

4.1.26. 4-Cyano-N-(4-Ethanesulfonylamino-3-Methoxyphenyl)Benzamide (22).

Yield 65.8%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.50 (s, 1H), 8.86 (s, 1H), 8.11 (d, 2H, J = 8.4), 8.05 (d, 2H, J = 8.4), 7.61 (d, 1H, J = 2.0), 7.34 (dd, 1H, J_1 = 2.0, J_2 = 8.4), 7.24 (d, 1H, J = 8.4), 3.83 (s, 3H), 3.00 (q, 2H, J = 7.2), 1.26 (t, 3H, J = 7.2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.54, 153.32, 139.32, 138.13, 132.95, 128.96, 127.11, 122.07, 118.76, 114.40, 112.83, 104.87, 56.14, 46.65, 8.47. DUIS-MS calculated for $C_{17}H_{17}N_3O_4S$, $[M-H]^-$: *m/z* 358.09, found *m/z* 357.9, Purity: 96.9%.

4.1.27. N-(4-(Ethylsulfonamido)-3-Methoxylphenyl)-3-(Trifluoromethyl)-Benzamide (23).

Yield 62.3%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.50 (s, 1H), 8.85 (s, 1H), 8.30 (s, 1H), 7.28 (d, 1H, J = 7.6), 7.99 (d, 1H, J = 7.6), 7.81 (t, 1H, J = 7.6), 7.60 (s, 1H), 7.36 (d, 1H, J = 8.8), 7.25 (d, 1H, J = 8.8), 3.84 (s, 3H), 3.01 (q, 2H, J = 7.2), 1.26 (t, 3H, J = 7.2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.44, 153.34, 138.20, 136.18, 132.28, 130.23, 130.18, 129.86, 129.53, 129.22 (q, 1C), 128.68 (m, 1C), 127.14, 128.52, 125.81, 123.10, 120.38 (q, CF_3), 124.65 (m, 1C), 122.00, 112.91, 104.97, 56.15, 46.65, 8.48. DUIS-MS calculated for $C_{17}H_{17}F_3N_2O_4S$, $[M-H]^-$: *m/z* 401.08, found *m/z* 400.9. Purity: 97.8%.

4.1.28. 4-Chloro-N-(4-(ethylsulfonamido)-3-methoxylphenyl)-3-nitrobenzamide (24).

Yield 69.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.56 (s, 1H), 8.86 (s, 1H), 8.64 (d, 1H, J = 2.0), 8.27 (dd, 1H, J_1 = 8.4, J_2 = 2.0), 7.99 (d, 1H, J = 8.4), 7.57 (d, 1H, J = 1.6), 7.34 (dd, 1H, J_1 = 1.6, J_2 = 8.8), 7.26 (s, 1H, J = 8.8), 3.83 (s, 3H), 3.01 (q, 2H, J = 7.2), 1.26 (t, 3H, J = 7.2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.86, 152.33, 147.87, 137.90, 135.27, 133.28, 132.47, 128.60, 127.15, 125.27, 122.20, 112.90, 104.90, 56.15, 45.67, 8.48. DUIS-MS calculated for $C_{16}H_{16}ClN_3O_6S$, $[M-H]^-$: *m/z* 412.04, found *m/z* 411.9. Purity: 96.7%.

4.1.29. N-(4-(Ethylsulfonamido)-3-Methoxylphenyl)-3,5-Bis (Trifluoromethyl)Benzamide (25).

Yield 62.4%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.68 (s, 1H), 8.88 (s, 1H), 8.62 (s, 2H), 8.40 (s, 1H), 7.57 (d, 1H, J = 2.0), 7.35 (q, 1H, J_1 = 2.0, J_2 = 8.6), 7.28 (d, 1H, J = 8.6), 3.84 (s, 3H), 3.01 (q, 2H, J = 7.3), 1.26 (t, 3H, J = 7.3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 162.93, 153.30, 137.77, 137.51, 131.50, 131.17, 130.84, 130.51 (q, 2C), 129.01 (m, 2C), 127.68, 124.96, 122.25, 119.53 (q, $2CF_3$), 127.03, 125.67 (m, 1C), 122.38, 113.09, 105.11, 56.20, 46.72, 8.48. DUIS-MS calculated for $C_{18}H_{16}F_6N_2O_4S$, $[M-H]^-$: *m/z* 469.07, found *m/z* 468.9. Purity: 98.2%.

4.1.30. 4-Chloro-N-(3-Methoxy-4-Trifluoromethanesulfonylamino-Phenyl)-B enzamide (26).

Yield 45.5%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.14 (s, 1H), 10.44 (s, 1H), 8.00 (d, 2H, J = 8.4), 7.66 (s, 1H), 7.64 (d, 2H, J = 8.4), 7.40 (d, 1H, J = 8.4), 7.22 (d, 1H, J = 8.4), 3.84 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.06, 155.45, 140.66, 137.09, 133.86, 130.11 (2C), 129.72, 128.99 (2C), 118.03, 112.58, 104.76, 56.23. DUIS-MS calculated for $C_{15}H_{12}ClF_3N_2O_4S$, $[M-H]^-$: *m/z* 407.01, found *m/z* 406.9. Purity: 97.4%.

4.1.31. 4-Cyano-N-(3-Methoxy-4-Trifluoromethanesulfonylamino-Phenyl) Benzamide (27).

Yield 45.0%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.22 (s, 1H), 10.60 (s, 1H), 8.12 (d, 2H, J = 8.4), 8.05 (d, 2H, J = 8.4), 7.66 (d, 1H, J = 1.6), 7.40 (dd, 1H, J_1 = 1.6, J_2 = 8.4), 7.24 (d, 1H, J = 8.4), 3.84 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.80, 155.46, 140.41, 139.19, 132.99 (2C), 129.78, 129.01 (2C), 118.75, 114.51, 112.66, 107.67, 104.79, 56.25. DUIS-MS calculated for $C_{16}H_{12}F_3N_3O_4S$, $[M-H]^-$: *m/z* 398.04, found *m/z* 397.9. Purity: 96.2%.

4.1.32. N-(3-Methoxy-4-Trifluoromethanesulfonylamino-Phenyl)-3-Trifluoromethyl-Benzamide (28).

Yield 45.8%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.21 (s, 1H), 10.58 (s, 1H), 8.30 (s, 1H), 8.28 (d, 1H, J = 7.6), 8.00 (d, 1H, J = 7.6), 7.82 (t, 1H, J = 7.6), 7.65 (s, 1H), 7.42 (d, 1H, J = 8.4), 7.25 (d, 1H, J = 8.4), 3.34 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.69, 155.47, 140.49, 138.52, 136.07, 132.03, 131.07, 130.78, 130.27, 129.74, 129.24 (q, 1C), 128.85, 128.47 (m, 1C), 124.74 (m, 1C), 112.75, 104.91, 56.27. DUIS-MS calculated for $C_{16}H_{12}F_6N_2O_4S$, $[M-H]^-$: *m/z* 441.03, found *m/z* 440.9. Purity: 98.7%.

4.1.33. 4-Chloro-N-(3-Methoxy-4-Trifluoromethanesulfonylamino-Phenyl)-3-Nitro-Benzamide (29).

Yield 50.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.22 (s, 1H), 10.66 (s, 1H), 8.65 (s, 1H), 8.28 (d, 1H, J = 8.0), 8.00 (d, 1H, J = 8.0), 7.63 (s, 1H), 7.41 (d, 1H, J = 8.4), 7.26 (d, 1H, J = 8.4), 56.29 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ:163.11, 155.47, 147.88, 140.19, 135.16, 133.32, 132.49, 129.76, 128.72, 125.32, 118.48, 112.76, 104.90, 56.29. DUIS-MS calculated for $C_{15}H_{11}ClF_3N_3O_6S$, $[M-H]^-$: *m/z* 452.01, found *m/z* 451.9. Purity: 97.8%.

4.1.34. N-(3-Methoxy-4-Trifluoromethanesulfonylamino-Phenyl)-3,5-Bis-Trifluoromethyl-Benzamide (30).

Yield 52.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.29 (s, 1H), 10.77 (s, 1H), 8.62 (s, 2H), 8.40 (s, 1H), 7.63 (s, 1H), 7.42 (d, 1H, J = 8.6), 7.28 (d, 1H, J = 8.6), 3.86 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.19, 155.47, 140.14, 137.40, 131.51, 131.18, 130.95, 130.52 (q, 2C), 129.79, 129.07 (m, 2C), 125.70 (m, 1C), 127.66, 124.94, 122.23, 119.52 (q, $2CF_3$), 118.53, 112.90, 105.02, 56.30. DUIS-MS calculated for $C_{17}H_{11}F_9N_2O_4S$, $[M-H]^-$: *m/z* 509.03, found *m/z* 508.9, Purity: 97.7%.

4.1.35. 4-Chloro-N-(3-Chloro-4-(MethylsulfonamidoPhenyl)Benzamide (31).

Yield 56.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.49 (s, 1H), 9.41 (s, 1H), 8.06 (s, 1H), 7.99 (d, 2H, J = 8.4), 7.71 (d, 1H, J = 8.4), 7.64 (d, 2H, J = 8.4), 7.44 (d, 1H, J = 8.4), 3.03 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.10, 138.75, 137.20, 133.62, 130.14 (2C), 130.09, 129.77, 129.22, 129.03 (2C), 212.42, 119.97, 41.31. DUIS-MS calculated for $C_{14}H_{12}Cl_2N_2O_3S$, $[M-H]^-$:356.99, found 356.8. Purity: 98.9%.

4.1.36. 4-Cyano-N-(3-Chloro-4-(MethylsulfonamidoPhenyl)Benzamide (32).

Yield 66.8%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.66 (s, 1H), 9.43 (s, 1H), 8.12 (d, 2H, J = 8.4), 8.07 (s, 1H), 8.06 (d, 2H, J = 8.4), 7.71 (d, 1H, J = 8.4), 7.46 (d, 1H, J = 8.4), 3.04 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.82, 138.94, 138.46, 133.01 (2C), 130.05, 129.19, 129.04 (2C), 121.51, 120.05, 118.72, 114.59, 99.99, 41.32. DUIS-MS calculated for $C_{15}H_{12}ClN_3O_3S$, $[M-H]^-$: *m/z* 348.02, found *m/z* 347.9, Purity: 98.7%.

4.1.37. N-(3-Chloro-4-(MethylsulfonamidoPhenyl)-3-(Trifluoromethyl) Benzamide (33).

Yield 69.8%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.84 (s, 1H), 9.45 (s, 1H), 8.34 (s, 1H), 8.11 (s, 1H), 7.99 (d, 1H, J = 6.8), 7.80 (m, 3H), 7.46 (d, 1H, J = 8.4), 3.04 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.71, 138.72, 135.71, 132.55, 130.19, 130.16, 129.84, 129.52, 129.21 (q, 1C), 130.13, 129.93, 129.11, 128.85 (m, 1C), 125.00 (m, 1H), 121.76, 120.24, 41.29. DUIS-MS calculated for $C_{15}H_{12}ClF_3N_2O_3S$, $[M-H]^-$: *m/z* 391.01, found *m/z* 390.9. Purity: 98.7%.

4.1.38. 4-Chloro-N-(3-Chloro-4-(methylsulfonamido)Phenyl)-3-Nitrobenzamide (34).

Yield 68.8%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.72 (s, 1H), 9.45 (s, 1H), 8.65 (s, 1H), 8.27 (d, 1H, J = 8.4), 8.05 (s, 1H), 8.00 (d, 1H, J = 8.4), 7.70 (d, 1H, J = 8.8), 7.47 (d, 1H, J = 8.8), 3.04 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.13, 147.88, 138.24, 134.91, 133.35, 132.53, 130.21, 130.03, 129.16, 128.93, 125.35, 121.60, 120.12, 41.34. DUIS-MS calculated for $C_{14}H_{11}Cl_2N_3O_5S$, $[M-H]^-$: *m/z* 401.97, found *m/z* 401.8. Purity: 98.6%.

4.1.39. N-(3-Chloro-4-(Methylsulfonamido)Phenyl)-3,5-Bis (Trifluoromethyl)Benzamide (35).

Yield 51.2%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.80 (s, 1H), 9.45 (s, 1H), 8.62 (s, 2H), 8.40 (s, 1H), 8.05 (s, 1H), 7.73 (d, 1H, J = 8.8), 7.50 (d, 1H, J = 8.8), 3.05 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.21, 138.14, 137.20, 131.54, 131.21, 130.88, 130.55 (q, 2C) 130.33, 130.00, 129.11, 129.07 (m, 2C), 127.64, 124.93, 122.23, 119.51 (q, $2CF_3$), 125.83 (m, 1C), 121.80, 120.26, 40.08. DUIS-MS calculated for $C_{16}H_{11}ClF_6N_2O_3S$, $[M-H]^-$: *m/z* 459.00, found *m/z* 458.8. Purity: 99.2%.

4.1.40. 4-Chloro-N-(3-Chloro-4-(Ethylsulfonamido)Phenyl)Benzamide (36).

Yield 66.3%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.48 (s, 1H), 9.38 (s, 1H), 8.05 (s, 1H), 7.99 (d, 2H, J = 8.0), 7.69 (d, 1H, J = 8.6), 7.63 (d, 2H, J = 8.0), 7.45 (d, 1H, J = 8.6), 3.12 (q, 2H, J = 7.2), 1.30 (t, 3H, J = 7.2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.07, 138.57, 137.19, 133.63, 130.14, 129.87, 129.83, 129.02, 121.40, 119.96, 99.99, 47.61, 8.56. DUIS-MS calculated for $C_{15}H_{14}Cl_2N_2O_3S$, $[M-H]^-$: *m/z* 371.00, found *m/z* 370.9. Purity: 98.1%.

4.1.41. 4-Cyano-N-(3-Chloro-4-(Ethylsulfonamido)Phenyl)Benzamide (37).

Yield 62.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.65 (s, 1H), 9.41 (s, 1H), 8.06 (s, 1H), 8.11 (d, 2H, J = 8.0), 8.05 (d, 2H, J = 8.0), 7.69 (d, 1H, J = 8.6), 7.46 (d, 1H, J = 8.6), 3.13 (q, 2H, J = 7.2), 1.29 (t, 3H, J = 7.2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.80, 138.94, 138.28, 133.00 (2C), 130.16, 129.79, 129.04 (2C), 128.98, 121.48, 120.05, 118.72, 114.60, 47.63, 8.56. DUIS-MS calculated for $C_{16}H_{14}ClN_3O_3S$, $[M-H]^-$: *m/z* 362.04, found *m/z* 361.9. Purity: 97.8%.

4.1.42. N-(3-Chloro-4-(Ethylsulfonamido)Phenyl)-3-(Trifluoromethyl) Benzamide (38).

Yield 62.5%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.66 (s, 1H), 9.41 (s, 1H), 8.31 (s, 1H), 8.28 (d, 1H, J = 7.4), 8.06 (s, 1H), 8.00 (d, 1H, J = 7.4), 7.81 (t, 1H, J = 7.4), 7.72 (d, 1H, J = 8.6), 7.47 (d, 1H, J = 8.6), 3.13 (q, 2H, J = 7.2), 1.30 (t, 3H ,J = 7.2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.68, 138.38, 135.82, 132.38, 130.29, 130.08, 130.21, 129.89, 129.57, 129.25 (q, 1C), 129.80, 128.97, 128.88 (m, 1C), 128.49, 125.78, 123.07, 120.36 (q, CF_3), 124.79 (m), 121.58, 120.11, 47.63, 8.56. DUIS-MS calculated for $C_{16}H_{14}ClF_3N_2O_3S$, $[M-H]^-$: *m/z* 405.03, found *m/z* 404.9. Purity: 99.1%.

4.1.43. 4-Chloro-N-(3-Chloro-4-(Ethylsulfonamido)-Phenyl)-3-Nitrobenzamide (39).

Yield 67.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.70 (s, 1H), 9.42 (s, 1H), 8.64 (s, 1H), 8.27 (d, 1H, J = 8.4), 8.03 (s, 1H), 8.00 (d, 1H, J = 8.4), 7.69 (d, 1H, J = 8.8), 7.48 (d, 1H, J = 8.8), 3.13 (q, 2H, J = 7.2), 1.30 (t, 3H, J = 7.2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.11, 147.88, 138.06, 134.92, 133.34, 132.53, 130.31, 129.77, 128.97, 128.82, 125.35, 121.55, 120.10, 47.65, 8.56. DUIS-MS calculated for $C_{15}H_{13}Cl_2N_3O_5S$, $[M-H]^-$: *m/z* 415.99, found *m/z* 415.8. Purity: 98.8%.

4.1.44. N-(3-Chloro-4-(Ethylsulfonamido)Phenyl)-3,5-Bis (Trifluoromethyl)Benzamide (40).

Yield 54.1%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.81 (s, 1H), 9.43 (s, 1H), 8.61 (s, 2H), 8.38 (s, 1H), 8.03 (s, 1H), 7.70 (d, 1H, J = 8.6), 7.49 (d, 1H, J = 8.6), 3.14 (q, 2H, J = 7.2), 1.30 (t, 3H, J = 7.2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.18, 137.95, 137.16, 131.54, 131.20, 130.87, 130.54 (q, 2C), 130.45, 129.73, 129.06 (m,2C), 128.89, 125.78 (m,1C), 127.62, 124.91, 122.20, 119.48 (q, $2CF_3$), 121.75, 120.24, 47.67, 8.53. DUIS-MS calculated for $C_{17}H_{13}ClF_6N_2O_3S$, $[M-H]^-$: *m/z* 473.02, found *m/z* 472.9. Purity: 97.2%.

4.1.45. 4-Chloro-N-(3-Chloro-4-(Trifluoromethylsulfonamido)-Phenyl)-Benzamide (41).

Yield 48.2%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.72 (s, 1H), 8.11 (d, 2H, J = 8.4), 8.10 (s, 1H), 8.06 (d, 2H, J = 8.4), 7.75 (d, 1H, J = 8.8), 7.46 (d, 1H, J = 8.8). ^{13}C NMR (100 MHz, DMSO-d_6) δ:164.96, 139.66, 138.87, 133.02 (2C), 131.73, 130.44, 129.08 (2C), 121.50, 120.08, 118.71, 114.66. DUIS-MS calculated for $C_{14}H_9Cl_2F_3N_2O_3S$, $[M-H]^-$: *m/z* 410.96, found *m/z* 410.8. Purity: 98.2%.

4.1.46. 4-Cyano-N-(3-Chloro-4-(Trifluoromethylsulfonamido)-Phenyl)-Benzamide (42).

Yield 50.5%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.70 (s, 1H), 8.11 (d, 2H, J = 8.4), 8.08 (d, 1H, J = 2.0), 8.06 (d, 2H, J = 8.4), 7.73 (dd, 1H, J_1 = 8.8, J_2 = 2.0), 7.45 (d, 1H, J = 8.8). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.92, 138.89, 137.63, 133.02 (2C), 131.55, 130.21, 125.35, 129.07 (2C), 121.49, 120.06, 118.71, 114.63. DUIS-MS calculated for $C_{15}H_9ClF_3N_3O_3S$, $[M-H]^-$: *m/z* 401.99, found *m/z* 401.8. Purity: 97.2%.

4.1.47. N-(3-Chloro-4-(Trifluoromethylsulfonamido)-Phenyl)-3-(Trifluoromethyl)-Benzamide (43).

Yield 49.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.73 (s, 1H), 8.31 (s, 1H), 8.28 (d, 1H, J = 7.6), 8.11 (s, 1H), 8.00 (d, 1H, J = 7.6), 7.81 (t, 1H, J = 7.6), 7.78 (d, 1H, J = 8.8), 7.46 (d, 1H, J = 8.8). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.85, 139.87, 135.73, 132.42, 131.80, 130.54, 130.31, 130.20, 129.89, 129.58, 129.27 (q, 1C), 128.96 (m, 1C), 124.83 (m, 1C), 128.47, 125.76, 123.05, 120.34 (q, CF_3), 121.58, 120.13, 118.66. DUIS-MS calculated for $C_{15}H_9ClF_6N_2O_3S$, $[M-H]^-$: *m/z* 444.98, found *m/z* 444.8. Purity: 98.6%.

4.1.48. 4-Chloro-N-(3-Chloro-4-(Trifluoromethylsulfonamido)Phenyl)-3-Nitrobenzamide (44).

Yield 49.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.79 (s, 1H), 8.645 (s, 1H), 8.27 (d, 1H, J = 8.4), 8.08 (s, 1H), 8.10 (d, 1H, J = 8.4), 7.74 (d, 1H, J = 8.8), 7.47 (d, 1H, J = 8.8). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.29, 147.87, 139.51, 134.83, 133.39, 132.54, 131.78, 130.54, 128.91, 125.41, 121.89, 121.58, 120.14. DUIS-MS calculated for $C_{14}H_8Cl_2F_3N_3O_5S$, $[M-H]^-$: *m/z* 455.94, found *m/z* 455.8. Purity: 97.8%.

4.1.49. N-(3-Chloro-4-(Trifluoromethylsulfonamido)Phenyl)-3,5-Bis (Trifluoromethyl)Benzamide (45).

Yield 48.8%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.85 (s, 1H), 8.61 (s, 2H), 8.40 (s, 1H), 8.07 (s, 1H), 7.75 (d, 1H, J = 8.8), 7.48 (d, 1H, J = 8.8). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.34, 137.16, 131.63, 131.54, 131.21, 130.78, 130.54 (q, 2C), 130.87, 130.31, 129.09 (m, 2C), 125.87 (m, 1C), 127.63, 124.92, 122.21, 119.49 (q, $2CF_3$), 121.98, 121.75, 120.25. DUIS-MS calculated for $C_{16}H_8ClF_9N_2O_3S$, $[M-H]^-$: *m/z* 512.97, found *m/z* 512.8. Purity: 97.3%.

4.1.50. 4-Chloro-N-(3-Fluoro-4-(Methylsulfonamido)Phenyl)Benzamide (46).

Yield 52.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.57 (s, 1H), 9.48 (s, 1H), 7.99 (d, 2H, J = 8.0), 7.84 (d, 1H, J = 12.8), 7.64 (d, 2H, J = 8.0),

7.54, (d, 1H, J = 8.6), 7.37 (t, 1H, J = 8.6), 3.01 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.09, 157.64, 155.21 (1C), 138.87, 138.77 (1C), 137.17, 133.69, 130.14 (2C), 129.02 (2C), 128.43, 120.47, 120.34 (1C), 116.75, 116.72 (1C), 108.45, 108.20 (1C), 40.07. DUIS-MS calculated for $C_{14}H_{12}ClFN_2O_3S$, $[M-H]^-$: *m/z* 341.02, found *m/z* 340.9. Purity: 96.8%.

4.1.51. 4-Cyano-N-(3-Fluoro-4-(Methylsulfonamido)Phenyl)Benzamide (47).

Yield 48.9%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.68 (s, 1H), 9.51 (s, 1H), 8.11 (d, 2H, J = 8.4), 8.05 (d, 2H, J = 8.4), 7.85 (d,1H, J = 12.4), 7.54 (d, 1H, J = 8.8), 7.39 (t, 1H, J = 8.8), 3.02 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.82, 157.56, 155.13 (1C), 139.00, 138.55, 138.45 (1C), 133.00 (2C), 129.04 (2C), 128.38, 120.79, 120.66 (1C), 118.72, 116.84, 116.81 (1C), 114.57, 108.54, 108.29 (1C), 56.50. DUIS-MS calculated for $C_{15}H_{12}FN_3O_3S$, $[M-H]^-$: *m/z* 332.05, found *m/z* 332.0. Purity: 98.8%.

4.1.52. N-(3-Fluoro-4-(Methylsulfonamido)Phenyl)-3-(Trifluoromethyl) Benzamide (48).

Yield 43.5%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.66 (s, 1H), 9.50 (s, 1H), 8.30 (s, 1H), 8.28 (d, 1H, J = 8.0), 8.00 (d, 1H, J = 8.0), 7.85 (d, 1H, J = 12.8), 7.81 (d, 1H, J = 8.0), 7.55 (d, 1H, J = 8.8), 7.40 (d, 1H, J = 8.8). 3.02 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.71, 157.59, 155.16 (1C), 138.63, 138.53(1C), 135.88, 132.36, 130.30, 130.21, 129.89, 129.57, 129.90 (q, 1C), 128.44, 125.78, 123.07, 120.36 (q, CF_3), 128.38, 124.73 (m, 1C), 123.07, 120.71, 120.58 (1C), 116.90, 116.87 (1C), 108.63, 108.38 (1C), 40.06. DUIS-MS calculated for $C_{15}H_{12}F_4N_2O_3S$, $[M-H]^-$: *m/z* 375.04, found *m/z* 374.8. Purity: 98.2%.

4.1.53. 4-Chloro-N-(3-Fluoro-4-(Methylsulfonamido)Phenyl)-3-Nitrobenzamide (49).

Yield 42.6%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.73 (s, 1H), 9.521 (s, 1H), 8.64 (s, 1H), 8.27 (d, 1H, J = 8.4), 7.999 (d, 1H, J = 8.4), 7.83 (d, 1H, J = 12.8), 7,53 (d, 1H, J = 8.8), 7.40 (t, 1H, J = 8.8, 3.023 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.14, 157.52, 155.10 (1C), 149.89, 138.31, 138.21 (1C), 134.97, 133.34, 132.52, 128.80, 128.37, 125.35, 120.95, 120.92 (1C), 116.92, 116.56 (1C), 108.64, 108.40 (1C), 40.06. DUIS-MS calculated for $C_{14}H_{11}ClFN_3O_5S$, $[M-H]^-$: *m/z* 386.00, found *m/z* 385.8; Purity: 96.3%.

4.1.54. N-(3-Fluoro-4-(Methylsulfonamido)Phenyl)-3,5-Bis (Trifluoromethyl)Benzamide (50).

Yield 41.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.85 (s, 1H), 9.54 (s, 1H), 8.61 (s, 2H), 8.40 (s, 1H), 7.84 (d, 1H, J = 12.8), 7.54 (d, 1H, J = 8.8), 7.42 (d, 1H, J = 8.8), 3.03 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.22, 157.50, 155.06 (1C), 138.21, 138.11 (1C), 137.23, 131.51, 131.19, 130.86, 130.53 (q, 2C), 129.08 (m, 2C), 128.27, 127.65, 124.93, 122.22, 119.50 (q, $2CF_3$), 125.82 (m, 1C), 121.08, 120.95 (1C), 117.06, 117.04 (1C), 108.81, 108.56 (1C), 40.06. DUIS-MS calculated for $C_{16}H_{11}F_7N_2O_3S$, $[M-H]^-$: *m/z* 443.03, found *m/z* 442.8. Purity: 97.8%.

4.1.55. 4-Chloro-N-(3-Fluoro-4-(Ethylsulfonamido)Phenyl)Benzamide (51).

Yield 50.2%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.51 (s, 1H), 9.51 (s, 1H), 7.99 (d, 2H, J = 8.4), 7.83 (dd, 1H, J_1 = 2.0, J_2 = 12.8), 7.64 (d, 2H, J = 8.4), 7.52 (d, 1H, J = 8.8), 7.37 (t, 1H, J = 8.8), 3.08 (q, 2H, J = 7.2), 1.28 (t, 3H, J = 7.2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.08, 157.44, 155.01 (1C), 138.70, 138.60 (1C), 137.16, 133.68, 130.13, 129.02, 128.33, 120.46, 120.33 (1C), 116.74, 116.70 (1C), 108.38, 108.13 (1C), 46.70, 8.48. DUIS-MS calculated for $C_{15}H_{14}ClFN_2O_3S$, $[M-H]^-$: *m/z* 355.03, found *m/z* 354.9. Purity: 97.5%.

4.1.56. 4-Cyano-N-(3-Fluoro-4-(Ethylsulfonamido)Phenyl)Benzamide (52).

Yield 53.2%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.68 (s, 1H), 9.53 (s, 1H), 8.11 (d, 2H, J = 8.4), 8.05 (d, 2H, J = 8.4), 7.84(d, 1H, J = 12.8), 7.53 (d, 2H, J = 8.8), 7.39 (t, 1H, J = 8.8), 3.08 (q, 2H, J = 7.2), 1.28 (t, 3H, J = 7.2) . ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.80, 157.37, 154.94 (1C), 138.99, 138.39, 138.29 (1C), 133.00 (2C), 129.04 (2C), 128.25, 120.79, 120.65 (1C), 128.73, 116.84, 116.80 (1C), 114.56, 108.48, 108.23 (1C), 46.72, 8.48. DUIS-MS calculated for $C_{16}H_{14}FN_3O_3S$, $[M-H]^-$: *m/z* 346.07, found *m/z* 345.8. Purity: 97.7%.

4.1.57. N-(3-Fluoro-4-(Ethylsulfonamido)Phenyl)-3-(Trifluoromethyl) Benzamide (53).

Yield 52.3%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.66 (s, 1H), 9.531 (s, 1H), 8.29 (s, 1H), 8.27 (d, 1H, J = 7.2), 8.00 (d, 1H, J = 7.2), 7.83 (m, 2H), 7.53 (d, 1H, J = 8.0), 7.40 (d, 1H, J = 8.0), 3.09 (q, 2H, J = 7.0), 1.28 (t, 3H, J = 7.0). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.69, 157.39, 154.97 (1C), 138.48, 138.38(1C), 135.87, 132.36, 130.29, 130.21, 129.88, 129.56, 129.24 (q, 1C), 128.87 (m, 1C), 128.26, 124.73 (m, 1C), 120.71, 120.58 (1C), 116.89, 116.85 (1C), 108.57, 108.31 (1C), 46.74, 8.49. DUIS-MS calculated for $C_{16}H_{14}F_4N_2O_3S$, $[M-H]^-$: *m/z* 389.06, found *m/z* 388.9. Purity: 97.5%.

4.1.58. 4-Chloro-N-(3-Fluoro-4-(Ethylsulfonamido)Phenyl)-3-Nitrobenzamide (54).

Yield 52.5%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.75 (s, 1H), 9.55 (s, 1H), 8.64 (s, 1H), 8.27 (dd, 1H, J_1 = 2.0, J_2 = 8.4), 8.00 (d, 1H, J = 8.4), 7.83 (d, 1H, J = 12.8), 7.52 (dd, 1H, J_1 = 8.8, J_2 = 2.0), 7.40 (t, 1H, J = 8.8), 3.09 (q, 2H, J = 7.3), 1.28 (t, 3H, J = 7.3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.12, 157.33, 155.00 (1C), 147.87, 138.17, 138.07 (1C), 134.96, 133.35, 132.50, 128.78, 128.24, 128.23 (1C), 125.37, 120.94, 120.81 (1C), 116.91, 116.88 (1C), 109.58, 109.33 (1C), 46.766, 6.48. DUIS-MS calculated for $C_{15}H_{13}ClFN_3O_5S$, $[M-H]^-$: *m/z* 400.02, found *m/z* 399.9. Purity: 98.8%.

4.1.59. N-(3-Fluoro-4-(Ethylsulfonamido)Phenyl)-3,5-Bis (Trifluoromethyl)Benzamide (55).

Yield 47.9%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.87 (s, 1H), 9.56 (s, 1H), 8.62 (s, 2H), 8.40 (s, 1H), 7.84 (d, 1H, J = 12.8), 7.53 (d, 1H, J = 8.8), 7.42 (t, 1H, J = 8.8), 3.10 (q, 2H, J = 7.2), 1.28 (t, 3H, J = 7.2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.20, 157.30, 154.87 (1C), 138.07, 137.97 (1C), 137.22, 131.51, 131.18, 130.84, 130.54 (q, 2C), 129.08 (m, 2C), 128.17, 125.77 (m, 1C), 127.65, 124.93, 122.22, 119.50 ($2CF_3$), 121.07, 120.94 (1C), 117.07, 117.03 (1C), 108.76, 108.50 (1C), 46.80, 8.49. DUIS-MS calculated for $C_{17}H_{13}F_7N_2O_3S$, $[M-H]^-$: *m/z* 457.05, found *m/z* 456.8. Purity: 98.5%.

4.1.60. 4-Chloro-N-(3-Fluoro-4-(Trifluoromethylsulfonamido)Phenyl) Benzamide (56).

Yield 45.6%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 16.20 (s, 1H), 10.59 (s, 1H), 7.99 (d, 2H, J = 8.4), 7.87 (d, 1H, J = 12.8), 7.64 (d, 2H, J = 8.4), 7.58 (d, 1H, J = 8.4), 7.39 (t, 1H, J = 8.4). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.26, 159.04, 158.57 (1C), 140.28, 140.31, 140.13 (1C), 137.26, 133.59, 130.17 (2C), 129.04 (2C), 119.34, 119.14 (1C), 116.82, 116.79 (1C), 108.32, 108.07(1C). DUIS-MS calculated for $C_{14}H_9ClF_4N_2O_3S$, $[M-H]^-$: *m/z* 394.99, found *m/z* 393.8, Purity: 97.8%.

4.1.61. 4-Cyano-N-(3-Fluoro-4-(Trifluoromethylsulfonamido)Phenyl) Benzamide (57).

Yield 47.6%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.77 (s, 1H), 8.11 (d, 2H, J = 8.0), 8.06 (d, 2H, J = 8.0), 7.89 (d, 1H, J = 12.8), 7.59 (d, 1H, J = 8.8), 7.42 (t, 1H, J = 8.8). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.02, 158.67, 156.21 (1C), 138.89, 133.16, 133.14 (1C), 133.01 (2C), 130.31, 129.09 (2C), 121.94, 121.91 (1C), 118.70, 116.93, 116.90 (1C), 114.66, 108.40, 108.15 (1C). DUIS-MS calculated for

$C_{15}H_9F_4N_3O_3S$, $[M-H]^-$: *m/z* 386.02, found *m/z* 385.8. Purity: 97.9%.

4.1.62. N-(3-Fluoro-4-(Trifluoromethylsulfonamido)-Phenyl)-3-(Trifluoromethyl)Benzamide (58).

Yield 47.5%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.78 (s, 1H), 8.30 (s, 1H), 8.29 (d, 2H, J = 7.6), 8.00 (d, 1H, J = 7.6), 7.91 (d, 1H, J = 12.4), 7.83 (t, 1H, J = 7.6), 7.61 (d, 1H, J = 8.2), 7.42 (t, 1H, J = 8.2) . ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.90, 158.68, 156.23 (1C), 140.42, 140.34 (1C), 135.78, 132.42, 132.38, 130.30, 130.27, 130.22, 129.90, 129.58, 129.26 (q, 1C), 128.917 (m, 1C), 124.81 (m, 1C), 123.06, 122.49 (1C), 116.99, 116.96 (1C), 108.50, 108.25 (1C). DUIS-MS calculated for $C_{15}H_9F_7N_2O_3S$, $[M-H]^-$: *m/z* 429.01, found *m/z* 428.8. Purity: 98.3%.

4.1.63. 4-Chloro-N-(3-Fluoro-4-(Trifluoromethylsulfonamido)Phenyl)-3-Nitrobenzamide (59).

Yield 43.5%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.76 (s, 1H), 10.48 (s, 1H), 8.65 (s, 1H), 8.28 (dd, 1H, J_1 = 8.4, J_2 = 1.2), 8.00 (t, 1H, J = 8.0), 7.86 (d, 1H, J = 12.8), 7.61 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.34, 158.67, 156.22 (1C), 147.88, 140.24, 140.14 (1C), 134.87, 133.38, 132.54, 130.37, 128.90, 125.41, 121.89, 121.34 (1C), 117.01, 116.98 (1C), 108.50, 108.25 (1C). DUIS-MS calculated for $C_{14}H_8ClF_4N_3O_5S$, $[M-H]^-$: *m/z* 439.97, found *m/z* 438.8. Purity:98.1%.

4.1.64. N-(3-Fluoro-4-(Trifluoromethylsulfonamido)Phenyl)-3,5-Bis(Trifluoromethyl)Benzamide (60).

Yield 40.2%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.94 (s, 1H), 8.61 (s, 2H), 8.40 (s, 1H), 7.90 (d, 1H, J = 12.4), 7.59 (d, 1H, J = 8.6), 7.45 (t, 1H, J = 8.6). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.43, 158.67, 156.21 (1C), 140.17, 140.07 (1C), 137.15, 131.56, 131.19, 130.86, 130.53 (q, 2C), 130.35, 129.15, 129.13 (3C), 125.84 (m, 1C), 127.63, 124.91, 122.20, 119.49 (q, 2CF_3), 117.13, 117.10 (1C), 108.65, 108.40 (1C). DUIS-MS calculated for $C_{16}H_8F_{10}N_2O_3S$, $[M-H]^-$: *m/z* 497.00, found *m/z* 497.0. Purity: 98.2%.

4.2. Solubility assay

Compounds 5, 57 and 15′ were used in the determination of their log P values. This method follows the OECD test guidelines for the slow stir method, with slight modification due to the data collection method.[22, 23] The data was collected on a UV–Vis spectrophotometer Shimadzu UV-1280 and standard UV Quartz 10 mm cuvette was used. The chemical was purchased from VWR brand EMPLURA 1-octanol 99% purity. The Flask where equipped with Teflon coated magnetic stirrers and Coring PC420D stir plates where used. The 1-octanol and DDH_2O where placed into two separate containers containing both phases to become mutual saturated by allowing them to stir for 48 h. Then the two phases where separated by a separation funnel. Compounds 5, 57, and 15′ now were dissolved in 1-octanol pre-saturated with water. These stock solutions are then centrifuged at 5000 rpm for 5 min. The supernatant was taken to be the working solution. Take 5 mL working solution of each into new clear flask, and add identical volume water saturated with 1-octanol, then stir at room temperature for 48 h. After that, stop stirring; take two-phase solution out, centrifuge at 5000 rpm for 5 min to separate this two-phase system. Removal of the 1-octanol phase from the solution then transferred for UV spectrophotometry to achieve absorbance at the correct wavelength. According to the procedure, concentrations were determined by UV measurement so, equation written as [23]:

$$\text{Log P} = \log \frac{A_x}{A_0 - A_x}$$

where A_0 and A_x are the initial and final absorbance values of organic layer.

4.3. Cell culture

HEK293 kidney cells and mouse macrophage RAW264.7 cells were obtained from ATCC (Rockville, MD) and maintained in RPMI1640 medium supplemented with 10% fetal bovine serum (FBS), 2 mM L-Glutamine, 1 mM sodium pyruvate, and 100 U/mL penicillin-streptomycin. FBS was heat inactivated for 30 min at 56 °C. Mammalian cells were grown at 37 °C in a Heraeus water-jacketed incubator with 5% CO_2. Bloodstream form *T. b. brucei* Lister 427 cells were cultured in HMI-9 medium with 10% FBS at 37 °C in a Heraeus water-jacketed incubator with 7.5% CO_2. The 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)–2H-tetrazolium (MTS) reagents were ordered from Promega life science (Madison, WI). 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) was ordered from Sigma-Aldrich (Milwaukie, WI).

4.4. Mammalian cell viability analysis

The MTT assay was used to examine the effect of tubulin inhibitors on the growth of HEK 293 and RAW 264.7 cells in four replications. 3000 cells per well were seeded with RPMI1640 medium in 96-well flat-bottomed plates for 24 h and were then exposed to various concentrations of test compounds dissolved in DMSO (final concentration ≤ 0.1%) in medium for 48 h. Controls received DMSO at a same concentration as that in drug-treated cells. Cells were incubated in 200 μL of 0.5 mg/mL of MTT reagent diluted in fresh media at 37 °C for 3 h. Supernatants were removed from the wells, and the reduced MTT dye was solubilized with 200 μL/well DMSO. Absorbance at 570 nm was determined on a SpectraMax Plus384 spectrophotometer (Molecular Devices). Data obtained with quadruplication were normalized and fitted to a dose–response curve using GraphPad Prism v.5 (GraphPad).

4.5. T. brucei cell viability analysis

The (MTS) assay was used to examine the effect of tubulin inhibitors on *T. brucei* cell viability.[21] 5000 cells of *T. brucei* were seeded in 96 well plates and treated with 0.1% DMSO and tested agents at various concentrations for 48 h at 37 °C. Subsequently, 20 μL of MTS (5% PMS) from the CellTiter Cell Proliferation Assay (Promega) was added to 200 μL of *T. brucei* culture in each well and incubated at 37 °C for 3 h. Soluble formazan, produced by viable cells due to reduction of MTS, was measured at 490 nm with a SpectraMax Plus 384 spectrophotometer (Molecular Devices). Data obtained with quadruplication were normalized and fitted to a dose-response curve using GraphPad Prism v.5 (GraphPad).

4.6. T. brucei cell lysate preparation and western blot assay

T. brucei cells were incubated with 0.5% DMSO and different doses of compound **5** and compound **57** for 12 h. Cell pellets were harvested by centrifugation at 1500 rpm for 10 min at room temperature, washed twice with 1X TDB buffer (5 mM KCl, 80 mM NaCl, 1 mM $MgSO_4$, 20 mM Na_2HPO_4, 2 mM NaH_2PO_4, 20 mM glucose, pH 7.4) with protease inhibitor (Roche) and PMSF (1 mM, Amresco), and lysed with 30 μL of lysis buffer (80 mM Pipes, pH 6.8, 2 mM EGTA, 1 mM $MgCl_2$, 0.15% Triton X-100, 10% glycerol, protease inhibitor (Roche) and PMSF) at 4 °C for 5 min. The cell lysate was centrifuged at 15,000 g for 30 min at 4 °C, and the supernatant was transferred into a fresh Eppendorf tube. Pellets were re-suspended in 40 μL of lysis buffer (RIPA, Thermo), and the sample was boiled at 95 °C for 15 min. Protein lysates from equal number of cells (1.83×10^7, split into tubulin non-polymerized and polymerized fractions) were separated on 10% polyacrylamide gels by electrophoresis. Proteins were transferred onto PVDF membranes. Tubulin antibody (Prod # MA1-19162, Life technology) was used in the following western analysis.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmc.2019.02.049.

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