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Modification and Antichlamydial Activity Evaluation on Dysregulators of Cylindrical Proteases

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Modification and Antichlamydial Activity Evaluation on Dysregulators of Cylindrical Proteases

by

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I will move on with all your hopes and your suggestions, study more and try to do more.

ABSTRACT

Chlamydia trachomatis infection is the most commonly reported sexually transmitted disease in the United States and the world. This pathogen can cause long-term health problems including blindness, pelvic inflammatory disease (PID) and ectopic pregnancy, which can be life-threatening if left untreated. To this day, there is no chlamydia-specific drug on the market. The standard treatment uses broad-spectrum antibiotics, which may affect regular functions of the commensal microbiota and leads to the development of bacterial resistance. Recently, a series of compounds based on Activators of Self-Compartmentalizing Proteases (ACP) was reported to show antichlamydial activity. Based on that scaffold, we prepared 21 compounds by doing modifications on it. Biological evaluation studies show that those analogs can halt the growth of *Chlamydia trachomatis*. The diversity of compounds and antichlamydial activity results point us a direction for further developing selective anti-chlamydia drug.

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LIST OF ABBREVIATIONS

2-SP Media: 2-Sucrose-phosphate based transport medium

ACP: Activators of self-compartmentalizing protease

ADEP: Acyldepsipeptides

AZM: Azithromycin

CDC: Center for disease control and prevention

$CDCl_3$: Chloroform

C. trachomatis: *Chlamydia trachomatis*

ClpP: Caseinolytic protease proteolytic subunit

DCM: Dichloromethane

DIPEA: *N,N*-Diisopropylethylamine

DMEM: Dulbecco's modified eagle medium

DMSO: Dimethyl sulfoxide

Doxy: Doxycycline

EB: Elementary body

EtOAc: Ethyl acetate

Et_3N: Triethylamine

Et_2O: Diethyl ether

EtOH: Ethanol

FAS II: Type II fatty acid synthesis

FBS: Fetal bovine serum

G6-P: Glucose-6-phosphate

HBSS: Hanks' balanced salt solution

HCl: Hydrochloric acid

Hep-2 Cell: Human epithelial type 2 cell

HPLC: High-performance liquid chromatography

HRMS: High resolution mass spectrometry

IFA: Immunofluorescence assay

IFU: Inclusion forming unit

KOH: Potassium hydroxide

LiOH: Lithium hydroxide

MOMP: Major outer membrane protein

NMR: Nuclear magnetic resonance

OXONE: Potassium peroxymonosulfate

PBS: Phosphate-buffered saline

PID: Pelvic inflammatory disease

Ppm: Parts per million

PrOH: Propanol

PrONa: Sodium propanolate

PyBOP: Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate

RB: Reticulate body

SAR: Structure–activity relationship

STI: Sexually transmitted infection

TEA: Triethylamine

THF: Tetrahydrofuran

TLC: Thin layer chromatography

UNMC: University of Nebraska Medical Center

CHAPTER 1: INTRODUCTION

The Center for Disease Control and Prevention (CDC) has estimated 68 million total sexually transmitted infections (STIs), and 26 million new cases, in the United States in 2018. The treatment of these diseases costs $16 billion in direct medical costs to the USA health care system.[1] Among all the STIs, C*hlamydia trachomatis* is the most commonly reported bacterial STI in the USA with 1,758,668 chlamydial infections reported by the CDC in 2018.[2] This Gram-negative bacterium targets epithelial cells in urogenital tract and conjunctiva[3] and can cause genital and ocular disease[4-6] utilizing major histocompatibility complex class I (MHC I) molecules to present pathogenic antigens.[7] More significantly, it is associated with a higher risk of HIV infection.[8] Due to the nature of this disease, chronic and repeat infections are becoming common.[9] In addition, C*. trachomatis* can cause pelvic inflammatory disease (PID) and infertility, resulting from chronic asymptomatic infections particularly in females.[4] *Chlamydia* can also be passed on from mother to child during birth[10] because the infants contact with infected cervical secretions during vaginal delivery.[11] Those infected cervical secretions may contain *C. trachomatis* if a mother has already been infected.[11]

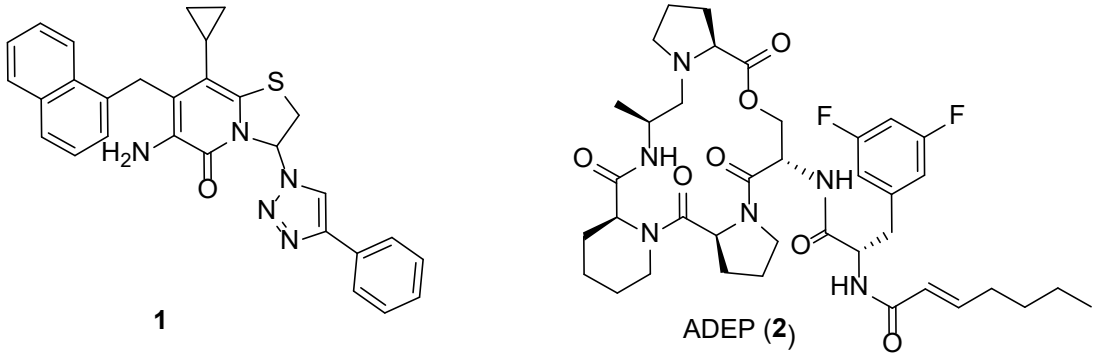
C. trachomatis has a biphasic developmental circle in which it cycles between two distinct morphological and different functional stages.[12] One is the non-dividing, infective elementary body (EB) , which is 0.25 to 0.30 μm in diameter, and can induce endocytosis upon exposure to the target cell to infect host cells. The second is the slightly bigger (0.50 – 0.60 μm in diameter) reticulate body (RB), which does not possess infectious ability.[12, 13] RBs can form membrane-bound vesicles within the host cells termed inclusion where the replication process takes place.[13] When several replication rounds finish, the RBs will turn back into EBs. Then, the host cells undergo an exocytosis process to release EBs to begin a new round of infection-replication.[14, 15]

At the moment, there is no specific *C. trachomatis* drug[16, 17] but a *Chlamydia* vaccine is under development.[17] Azithromycin (AZM) and doxycycline (Doxy) are the standard drugs utilized to treat this infection.[18] These antibiotics play a critical role in treating the disease but it has been reported that recurrent infections and treatment failure are occurring. For example, a high prevalence (>30%) of *C. trachomatis* infection has been reported in India.[19] Also, when using broad-spectrum antibiotics to treat females with STIs like *Chlamydia*, it was reported that the optimal state predominated by *Lactobacilliin* in human vagina may change to a variant polymicrobial state which link to a few of diseases.[20] A temporary decrease in diversity in vaginal microbiota was observed when treating with metronidazole(an alternative treatment for *Chlamydia*).[20]

Several novel approaches are under development for the treatment of *C. trachomatis*. For example, the researchers were able to develop molecules that affect the glucose-6-phosphate (G6-P) pathway, which is key in the energy uptake of *Chlamydia trachomatis*.[21, 22] This can be achieved with thiazolino 2-pyridone amides (**1, Figure 1**) which display promising inhibitory activity ($EC_{50} \leq 20$ nM) without affecting host cell or commensal bacteria viability.[21, 22] Also, type II fatty acid synthesis (FASII) pathway is essential for bacterial lipid biosynthesis. In *Chlamydia*, this pathway is indispensable for its growth. Gylfe's group focused on blocking the FAS II pathway to inhibit chlamydial growth.[23] They combined pharmacophores of *C. trachomatis* inhibitors with inhibitors of type III secretion which is critical in gram-negative bacteria to perform dual activity to study new antichlamydial agents.[24]

However, those drugs were not selective specifically to *Chlamydia*. It is reported that by targeting FAS II enzymes, drugs platensimycin and platencin showed potent activity against *Staphylococcus aureus, Enterococcus faecalis, Enterococcus faecium, and Streptococcus pneumoniae.*[25, 26] The FAS II pathway is also wildly represented in commensals like *Bifidobacterium* and *Streptococcus*.[27, 28] Therefore, blocking the FAS II pathway is also detrimental to bacteria within native microbiomes. Though FAS II

pathway may be a great target, the drugs which aiming FAS II pathway are not selectively targeting *Chlamydia*. Given the increasing rate of STIs and rising of general bacterial resistance because of the untargeted treatments, it is urgent to discover selective anti-chlamydia drugs to deal with the deleterious consequences of *C. trachomatis* infections.



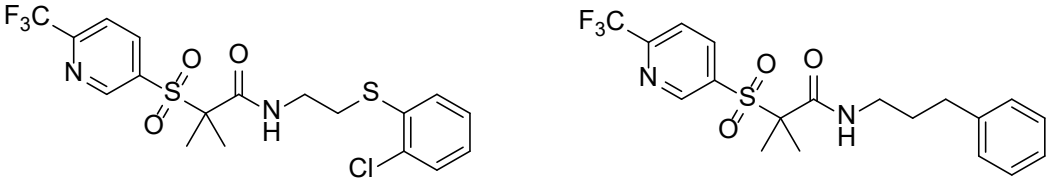
1 ADEP (**2**)

Figure 1 Structures of thiazolino 2-pyridone amides and ADEP compound

Cylindrical proteases play an indispensable role in cellular protein quality control and have been considered as a worth-to-pursue antimicrobial target.[29-31] Some studies have mentioned that disrupting the normal function of cylindrical protease can halt bacterial growth.[31-33] This is a consequence of the dysregulation of the tightly controlled protein turnover process.[34, 35] For example, a novel strategy against *Escherichia coli* was reported by Heike Brötz-Oesterhelt.[36] To affect the growth of this pathogen, the authors used molecules known as acyldepsipeptides (ADEP, **2**). Binding of ADEPs to caseinolytic proteases (ClpP) can activate uncontrolled proteolysis and leads inhibition of pathogen cell division and cell death as a result. Besides *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Haemophilus influenzae* and *Neisseria meningitidis* were also found to be sensitive to ClpP activation.[37-40] Despite the relatively good inhibitory activity, the ADEP still possesses several disadvantages such as nondrug-like structure, difficultly to modify, poor aqueous solubility, and a poor pharmacokinetic profile.[40-42]

Based on the aforementioned studies, Houry's laboratory used high-throughput

screening technology on ClpP to discover ADEP mimics. They found some structures that are able to inhibit bacterial growth, which they called Activators of Self-Compartmentalizing Proteases (ACP).[40] Although the ACPs make less contact in the ClpP binding pocket than ADEPs, they possess a similar mechanism of ClpP activation.[40] Noteworthy, one molecule ACP1b (**32**) showed an 8 ug/mL antibacterial activity against *Haemophilus influenzae.* It is indicated that the ACP can be further developed as an antibiotic targeting ClpP.



ACP1b (**32**) **2**

*Figure 2 Structure of ACP1b and compound **2***

As mentioned, ClpPs are essential for bacterial growth. In *C. trachomatis*, it also serves a critical role.[43] Recent work from the Ouellette group at UNMC identified 5 *clp* genes that generate 5 Clp proteins that regulated the developmental cycle of this organism.[43] Most importantly, the ClpP1 and ClpP2 serve as caseinolytic proteases. Then, the ClpC and ClpX are ATPase that can facilize the protein going into ClpP protease to be degraded, ClpB is a deaggregase.[43, 44] Starting with Walid's group discovery of ACP, the ACP1b and compound **2** were synthesized by our group and their activity against *Chlamydia trachomatis* were tested.[43, 45] ACP1b was able to decrease the number of inclusions, which indicated the ability to halt chlamydia growth.[43]

ClpP can be the novel target for treating *C. trachomatis*. As mention above, there are two distinct ClpP paralogs (ClpP1 and ClpP2) in *C. trachomatis* and each of them has an independent function.[43] By activating ClpP2, the chlamydial growth could be blocked. Also, in other bacteria like *Pseudomonas aeruginosa*, the ClpP system with 2 paralogs perform the distinct function contributing to virulence and fitness of the

bacterium.[46] So, it is noteworthy to consider ClpP as a novel target to halt bacteria growth especially *C. trachomatis* growth.

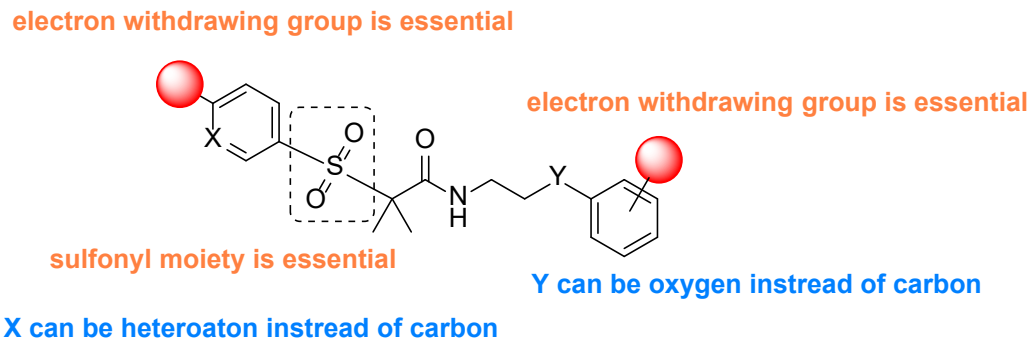


Figure 3 Preliminary SAR study from our group[45]

Building upon the preliminary SAR (**Figure 3**) [45] by our group, a new series of compounds was synthesized and tested. Various compounds showed antichlamydial activity. The results are helpful for the further development of new selective antichlamydial drugs.

CHAPTER 2: METHODS

Chemistry

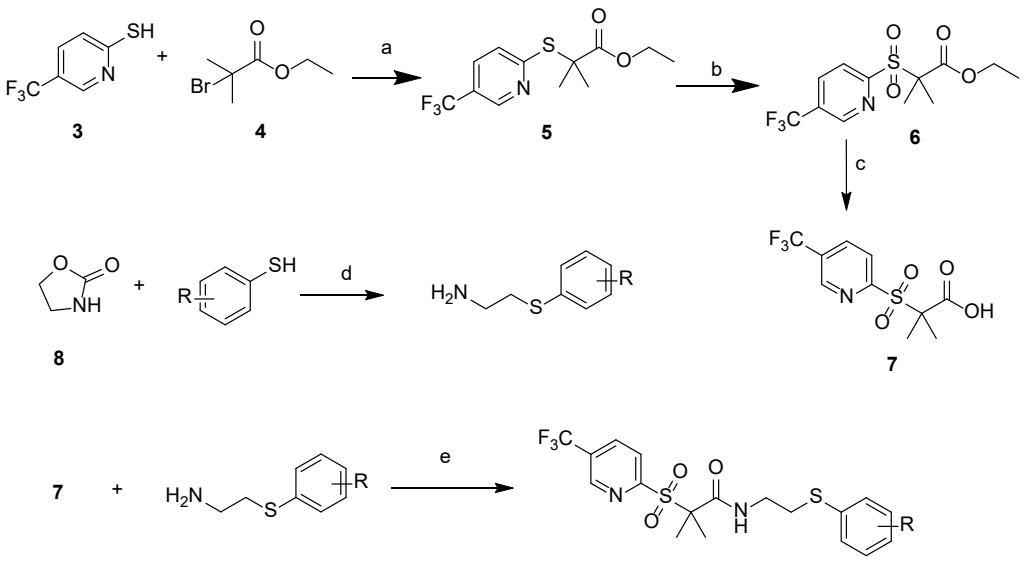
General

All reagents and solvents were purchased from common providers including TCI, Fisher, Acros, or Alfa Aesar. All air-sensitive reactions were protected under N_2 gas. All solvents needed to be used in moisture-sensitive reactions were dried and put under activated molecular sieves. Thin Layer Chromatography (TLC), which were performed on Merck silica gel IB2-F plates (2 cm x 7 cm, 0.25 mm thickness), were used to detect the progression of the reactions under UV light at 254nm or by potassium permanganate stain as indictor. Flash column chromatography was performed on Teledyne Isco CombiFlash Rf+ (UV). Nuclear magnetic resonance (NMR) experiments were done in a Bruker 500 MHz Advance III HD spectrometer at 500 and 125 MHz for ^{1}H and ^{13}C respectively with solvent peak as an internal standard. Multiplicates were showed by s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet), br (broad). Chemical shifts (δ) are described in parts per million (ppm), and coupling constants (*J*), in hertz. The solvent used in NMR was deuterated chloroform ($CDCl_3$) or dimethyl sulfoxide (DMSO- d_6). All final compounds going through biological test possessed above 95% purities, as confirmed on an Agilent 1220 Infinity LC equipped with a Kinetex 250 x 4.6 mm C-18 reversed-phase column using methanol and water as mobile phases. The wavelength for UV absorbance was set at 254 nm and a wash run was performed every ten samples. All tested compounds were preserved in a -20 ℃ refrigerator after synthesized before biological testing.

General Procedures to Prepare Carboxylic Acid Derivatives.

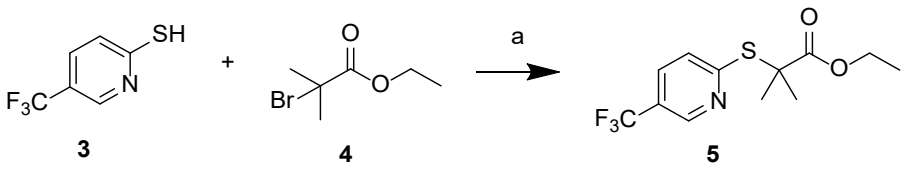
The whole synthetic route is shown below in **Figure 1**.

Scheme 1 General Synthetic Route of Tested Compounds



Reagents and conditions: (a) KOH, EtOH, reflux, overnight; (b) OXONE, dioxane-water (5:1), room temperature, overnight; (c) LiOH, THF-water (4:1), room temperature, overnight; (d) Na, 1-propyl alcohol, reflux, 6 h; (e) PyBOP, DIPEA, THF, room temperature, 1 h.

Scheme 2 Synthetic Step 1 to Make Acid Intermediate.



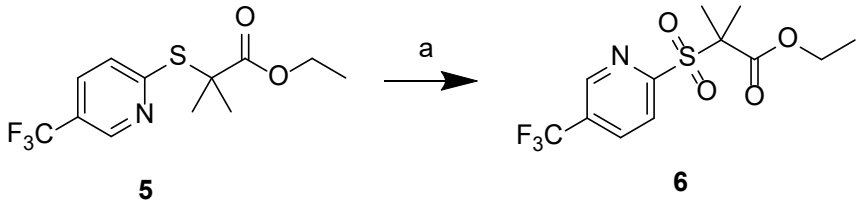
Reagents and conditions[43]: (a) KOH, EtOH, reflux, overnight.

Ethyl 2-methyl-2-((5-(trifluoromethyl)pyridin-2-yl)thio)propanoate (**5**):

5-(trifluoromethyl)pyridine-2-thiol (**3**, 1.00 g, 5.58 mmol) was dissolved in ethyl alcohol (20 mL) and potassium hydroxide pellets (0.47 g, 8.37 mmol) were added. After the KOH pellets were totally dissolved under stirring, ethyl 2-bromo-2-methylpropanoate (**4**, 1.10 g, 5.58 mmol) was added to the round bottom flask. The reaction mixture was stirred under refluxed overnight. After the confirmation of reaction completion by TLC, the flask containing the reaction mixture was cooled to room temperature. The white

inorganic salt was filtered off and washed with ethyl alcohol. Then, the reaction solution and the washes were combined and evaporated under vacuum to obtain a yellow oil. The yellow oil was dissolved in ethyl acetate (20 mL) and washed with water (3 x 20 mL) and brine (20 mL). After the organic solution was dried over sodium sulfate and filtered, evaporated under vacuum to afford desired yellow oil that did not need further purification. The yield of the reaction is above 90%.

Scheme 3 Synthetic Step 2 to Make Acid Intermediate.



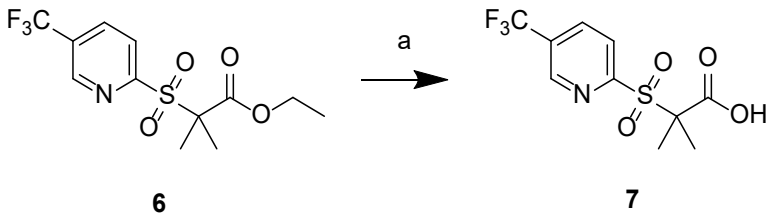
5 **6**

Reagents and conditions[43]: (a) OXONE, dioxane-water (5:1).

Ethyl 2-methyl-2-((5-(trifluoromethyl)pyridin-2-yl)sulfonyl)propanoate (**6**):

Ethyl 2-methyl-2-((5-(trifluoromethyl)pyridin-2-yl)thio)propanoate (**5**, 1.50 g, 5.11 mmol) was dissolved in dioxane-water, 5:1 (24 mL). Potassium peroxymonosulfate (OXONE) (12.3 g, 20.46 mmol) was added to the reaction mixture. The reaction mixture was stirred at room temperature and monitored by TLC. After 24 hours, the white suspension was filtered, the obtained white precipitate was washed with dioxane (3 x 10 mL). The reaction solution and the washes were combined and evaporated under vacuum. Water (15 mL) was added to the acquired white foam-like material. Then the reaction mixture was washed with dichloromethane (3 x 15 mL). The collected organic solution was dried with sodium sulfate, filtered and evaporated under vacuum to provide a transparent oil which will form white solid. The yield of this reaction is 62%.

Scheme 4 Synthetic Step 3 to Make Acid Intermediate.



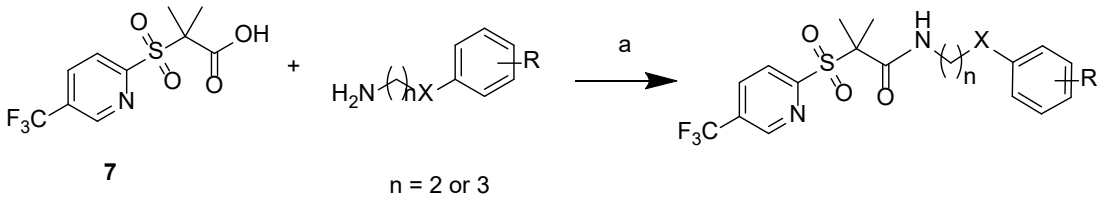
6 **7**

Reagents and conditions[43]: (a) LiOH, THF-water (4:1), room temperature, overnight.

2-methyl-2-((5-(trifluoromethyl)pyridin-2-yl)sulfonyl)propanoic acid (**7**):

The ethyl 2-methyl-2-((5-(trifluoromethyl)pyridin-2-yl)sulfonyl)propanoate (**6**, 1.00 g, 30.7 mmol), was dissolved into tetrahydrofuran-water, 4:1 (25 mL). Lithium hydroxide powder (0.147 mg, 6.15 mmol) was added into solution. The reaction mixture was stirred overnight at room temperature and stopped after confirmation of completion by TLC. The solvent was evaporated under vacuum and water (10 mL) was added. The suspension was washed with dichloromethane (20 mL) to remove any other byproducts and placed in an ice bath. A 1N hydrochloric acid solution was added drop-wise into the aqueous solution until the pH reached 2. The formed white precipitate was filtered and dried to afford the desired compound as white solid in a yield of no less than 40%.

Scheme 5 Coupling Step to Make Tested Compounds



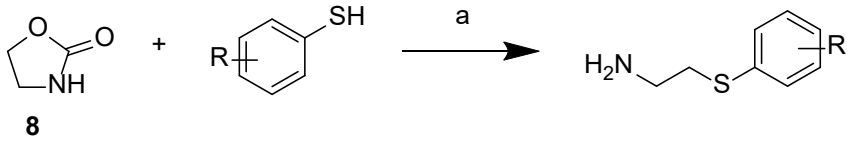
7 n = 2 or 3

Reagents and conditions[40]: (a) PyBOP, DIPEA, THF, room temperature, 1 h.

The 2-methyl-2-((5-(trifluoromethyl)pyridin-2-yl)sulfonyl)propanoic acid (**7**, 150 mg, 1.00 equivalent) was dissolved in THF (20 mL) and PyBOP (1.10 equivalents) was added followed by DIPEA (4.00 equivalents) dropwise. Then, the reaction solution was

stirred for 10 min. The amine derivatives (1.00 equivalent) was added at room temperature. After stirring for one hour, the reaction solvent was evaporated under vacuum and purified by flash column chromatography to afford pure compounds with gradient 0% - 70% ethyl acetate/hexane. The yield, physical properties and the spectral data are listed in Chapter 3.

Scheme 6 General Step to Make Amine Derivatives.



Reagents and conditions[47]: (a) Na, PrOH, reflux, 6 h.

Sodium (185 mg, 8.05 mmol) was dissolved in 1-propanol (20 mL). A benzenethiol derivative (1.00 equivalent) was added and stirred for 30 minutes at room temperature. Then oxazolidin-2-one (351 mg, 4.03 mmol) was added, and the reaction mixture was refluxed overnight. The reaction solution was cooled down to room temperature and the solvent was evaporated under vacuum. Water (20 mL) was added, and the solution was washed with diethyl ether (3 x 15 mL). The collected organic solution was dried with sodium sulfate, filtered and purified by silica chromatography with gradient 0% - 10% methanol/ dichloromethane to reach the yield of no less than 25%.

Biological Evaluation

General

All the flasks and bottles containing cells or solutions were autoclaved and kept under clean dry condition. All the cells infected or uninfected were cultured under consistent condition (37℃, 5% (v/v) CO_2) without significant change in HERAcell VIOS 160i CO_2 incubators (ThermoScientific). All the biological experiments procedure were done by single person to minimize the influence of changing people manipulate. 70% (v/v)

methanol was used to clean hands and all equipment every time before experiment done in the biological hood. All cells containing *Chlamydia* were disinfected before washing into the disposal. The cell line used in the experiments was human epithelial cell line HEp-2. The cell line was routinely propagated no longer than four days. Control groups were set in every experiment to control both influences on environment and solution. An Olympus CKX53 microscope with Olympus EP50 camara and a CoolLED's pE-300^{lite} LED illumination system was used to capture the fluorescent image.

Preparation of DMEM Media

The bottles containing media were disinfected before use. The Dulbecco's modified Eagle medium (DMEM; Gibco) with 2% L-glutamine and 10% fetal bovine serum (FBS; Gibco) was mixed well into the bottle without shaking hard. The mixed solution was kept in the refrigerator.

Split Cells

Before work, all the DMEM, phosphate buffered saline (PBS; Gibco) and trypsin were warmed up at 37° C for 30 min. The cell lines were analyzed under microscope to make sure that they were confluent, and the cell solution was without infection. We aspirated the culturing media under laminar flow with Pasteur pipette and then added PBS to wash and let cell shrink for next step. Shaked well to let PBS cover all surface of cell flask and aspirate it with new Pasteur pipette. Trypsin was added into flask to detach cell from wall and kept it for around 10 minutes. Hit the walls of flask with hand to let all cells separated from base of flask. New flask was prepared and labeled with proper experimental details, and then filled with DMEM media without bubble. Another amount of DMEM media was added into the old flask. The old flask was washed several times. Took enough amount of media containing cells into the new flask for further culturing.

Preparation of Compounds' Solutions

The synthesized compounds were weighed in the small vials from 1mg to 2mg, separately. Adding sterile dimethyl sulfoxide to let the concentration reach at 25 mg/mL. When adding drug later, 1 μL of DMSO solution with drug was added into 0.5 mL DMEM solution to give a 500 times dilution to let drug concentration reach 50 μg/mL.

Plate Cells

Before working, all the DMEM, PBS and trypsin were warmed up at 37° C for 30 min. Brought out the flask containing cell and checked cell lines under microscope to make sure the cell line is confluent, and the cell solution was clean without infection. Aspirated culturing media under laminar flow with Pasteur pipette and then added PBS to wash and let cell shrink for next step. Shaked well to let PBS cover all surface of cell flask and aspirated it with new Pasteur pipette. Trypsin was added into flask to detach cell from wall and kept it for around 10 minutes. Hit the walls of flask with hand to let all cells separated from base of flask. Transferred all the media solution into a new sterile centrifugation tube waiting for counting. In order to get 2 x 10^5 cell concentration in each plate well of the 24-well plate, the trypan blue dye assay was used to count the cell number and dilution factor. Then we diluted media containing cells and filled it with DMEM media for further plating. The media containing cells will be filled into 24 well plate for further testing.

Infect Cells and Add Compounds

Before cell work, all the DMEM, 2-sucrose-phosphate based transport medium (2-SP media) and HBSS were warmed up at 37° C for 30 min. In one Eppendorf tube, 2-SP media and *Chlamydia* stock solution media were mixed and then transferred into one centrifugation tube filled with DMEM media. Brought out the flask containing cell and checked cell lines under microscope to make sure the cell line was confluent, and the cell solution was clean without infection. Aspirated the media from flask and HBSS media was added into each well. Aspirated HBSS from each well then DMEM

containing *Chlamydia trachomatis* serovar L2 was added into each well. Then, the plate was incubated for 8 hours in the incubator at 37° C 5% (v/v) CO_2. Compounds were then added into each well in 50 μg/mL concentration according to preliminary testing.[45] For titration assay and reinfection assay, the compounds were added in diluted concentration.

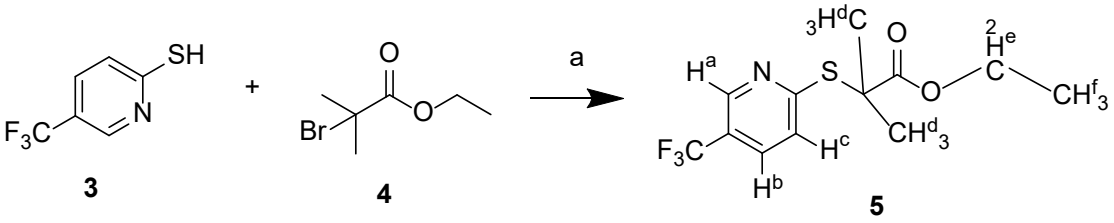
Fixing Cells

After 24 hours after infection, the plate was took out from the incubator and the media was aspirated. PBS azide solution was added into each well and aspirated. Methanol was followed to be added for 20 minutes to completely eradicate *Chlamydia* and other pathogens. PBS azide solution was added again for three times for further washing. Next, the primary goat anti-major outer membrane protein (MOMP; Meridian, Cincinnati, OH) (250 μl) was added into each well for one hour. Then the primary antibody was collected and each well was washed with PBS azide for three times. A donkey secondary antibodies (Invitrogen, Carlsbad, CA) labeled with Alexa488 was added for 1 hour. Then collected the secondary antibody and each well was washed with PBS azide solution for three times and filled with PBS azide. The images were waiting for further visualization under fluorescent microscope(Olympus, CKX53). 15 field of vision (FOV/well) were used at 20× magnification to count the inclusions under fluorescent microscope. [43, 48]

CHAPTER 3: RESULTS AND DISCUSSIONS

General Synthetic Steps

Scheme 7 Synthetic Step 1 to Make Acid Intermediate.



Reagents and conditions: (a) KOH, EtOH, reflux, overnight.

The preparation of the aryl-alkyl thioether (**Scheme 7**) occurs through a S_N2 mechanism. For this reaction, KOH was chosen as base; however, Na_2CO_3 can also be used although the reaction proceeds with a lower yield. The reaction proceeds better under reflux; i.e. at room temperature 30 hours need for completion but under reflux conditions, the procedure was completed overnight. After the disappearing of starting materials **3** and **4** as monitored by TLC, we cease the reaction and let it cool down to room temperature. A white precipitate appeared, most likely KBr that can be filtered off during workup. Then the organic solution was washed with water for three times to completely remove the inorganic salt. The whole reaction got an over 90% yield which does not need further purification. The result was confirmed with ^{1}HNMR. In **Figure 4**, we can see clearly that the singlet peak in 8.5 ppm is the H^a connected to the N atom, then the two-doublet peak also showed in aromatic region corresponding to H^b and H^c, respectively. Both the quartet peak at 4.1 ppm (H^e) and triplet peak at 1.2 ppm (H^f) obey “n+1” rule. Finally, the peak integrated as 6 in 1.7ppm was 6 H^d belonged to two methyl group.

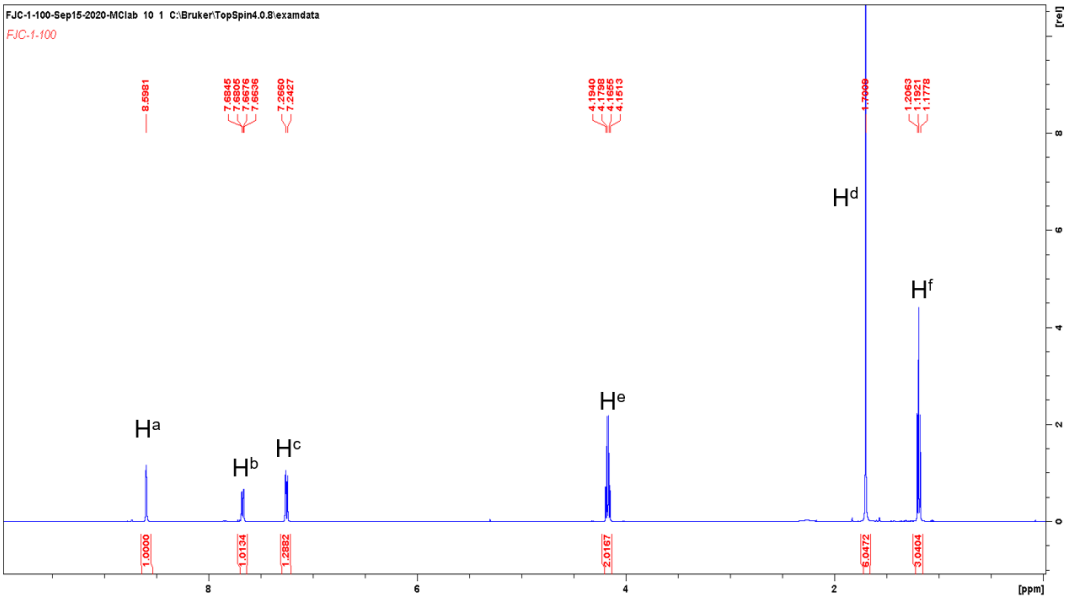
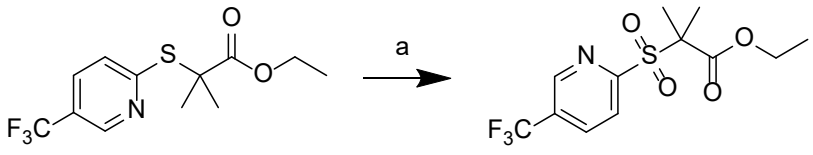


*Figure 4 [1]HNMR of compound **5***

Scheme 8 Synthetic Step 2 to Make Acid Intermediate.



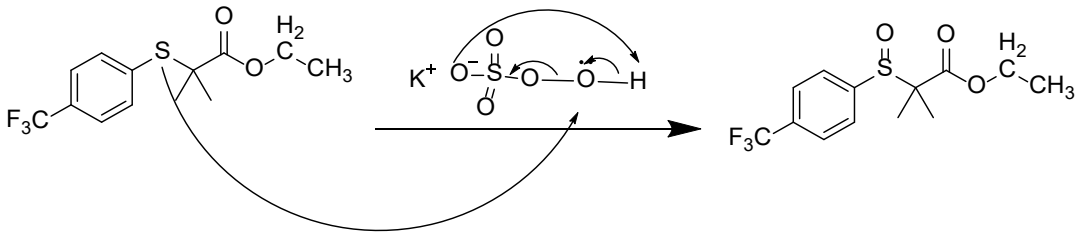
5 **6**

Reagents and conditions: (a) OXONE, dioxane-water (5:1), room temperature

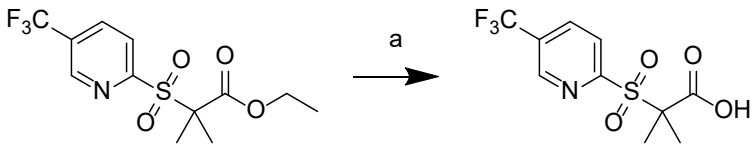
In **Scheme 8**, the reaction goes through an oxidation reaction with potassium peroxymonosulfate (OXONE) reagent. The reaction mechanism is showed in **Scheme 9**.[49] The oxygen atom on the right part of OXONE reagent will be deprotonated, then the lone pair on the sulfur atom will attack the oxygen, following by kicking off the rest of the molecule. 4 equivalents of oxidation reagent were used to let the reaction reach a yield of 62%. When using 2 equivalents of OXONE reagent and the same reaction time, the yield was 40%. The reason why mixture of dioxane and water was chosen as the reaction solvent is because the OXONE is slightly soluble in the organic solvent. Overnight reaction time was needed to finish this

reaction. Heating can help speeding up reaction but there was no significant improvement. The TLC plate showed two new spots and the disappearing of starting material when the reaction was finished. Two spots were belonged to sulfoxide and sulfone, respectively. The reaction needs to be continued if two spots are observed, or the yield of final acid derivatives will be lower. Washing with distilled water could remove inorganic salt and transparent oil was obtained without further purification. After ~10 minutes, the transparent oil crystallized as a white solid. The appearance of the solid is one way to assess whether the compound was made.

Scheme 9 Mechanism of Oxidation Reaction



Scheme 10 Synthetic Step 3 to Make Acid Intermediate.



6 **7**

Reagents and conditions: (a) LiOH, THF-water (4:1), room temperature, overnight

In **Scheme 10**, the reaction goes through a hydrolysis reaction using LiOH. As discussed in **Scheme 8**, the water was used as a solvent for the reason that LiOH will be better dissolved in water than in organic solvent. 8 hours was enough for this reaction to reach the yield of 55%, but overnight reaction time is preferred to reach the yield of 80%. After evaporating the reaction solvent, the water layer which contains the acid compound was washed with DCM to remove organic byproduct. The carboxylate compound was dissolved in water layer when the pH is high. After 1N HCl was added

into the reaction solution dropwise to let the solution's pH from 12 to 2, the compound will be precipitated as white crystal because it transformed into carboxylic acid form. During the first few drops of HCl, the white precipitate was appeared and vanished quickly. As the pH was getting lower, the white precipitate was accumulated in the solution. The flask was placed in the refrigerator for 1 hour to allow additional crystallization. After filtration, white crystals were obtained. The crystals were dried, and their structure was studied by ^{1}H NMR (**Figure 5**). The aromatic region integrated as 3 which present pyridine ring and the peak in 1.7ppm integrated as 6 represent two methyl group next to sulfone structure.

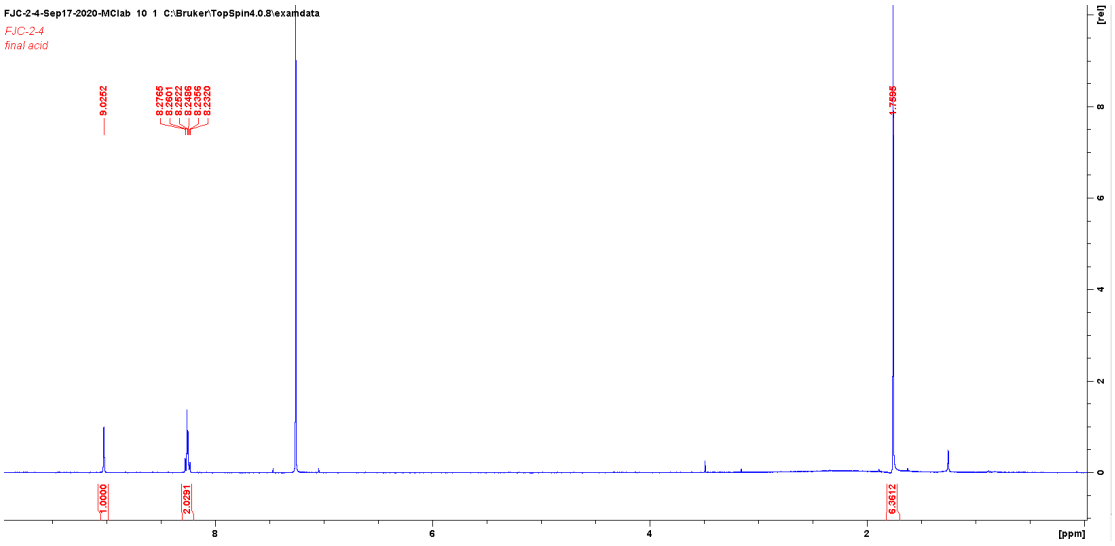
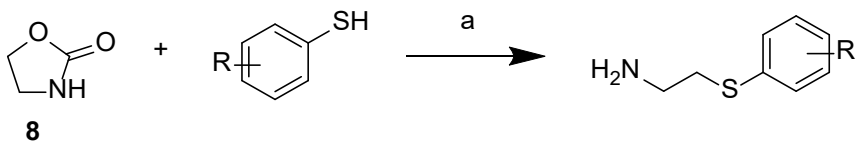


*Figure 5 ^{1}HNMR of compound **7***

Scheme 11 General Step to Make Amine Derivatives.



8

Reagents and conditions[47]: (a) Na, PrOH, reflux, 6 h.

In order to generate a proper base for this reaction, 2 equivalents of sodium were added into 1-propanol (20 mL). After the sodium got totally dissolved which means the PrONa was formed as a good base. Then 3 equivalents of benzenethiol were added

into the solution. The whole solution was stirred for 30 mins to let the base do deprotonation in thiol position. Finally, the reaction mixture was added 1 equivalent of oxazolidine-2-one (**8**) and the solution was heated under reflux overnight. The solvent selection was critical in this reaction. As shown in **Table 1**, only 1-proponal provided a good yield.

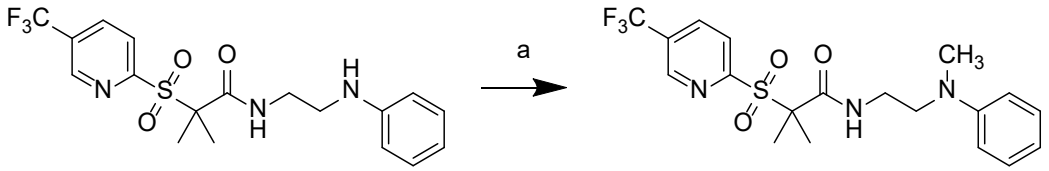
Table 1 Solvent Condition Optimization of Reaction for Amine Derivatives

solvent	yield %
Methanol	Trace
2-Propanol	Trace
1-Propanol	87%

Specific Compounds Synthetic Methods

22

*Scheme 12 Reaction Scheme to Compound **22***



12 **22**

Reagents and conditions: (a) NaH, DMF, 0℃ - room temperature, overnight

In order to obtain compound **22**, an *N*-methylation reaction method was utilized. From the theoretical point of view, we need one basic reagent to deprotonate the N atom, then we have our methylation reagent attacking that the N atom to get *N*-methylation. From **Table 2**, three different bases were used such as K_2CO_3, triethylamine (TEA) and NaH. NaH was best for this reaction because it is a stronger base than TEA and K_2CO_3. The reaction with NaH also achieved a better yield. Moreover, the selection of solvent was also essential for this reaction. As shown in **Table 2**, DMF has a better

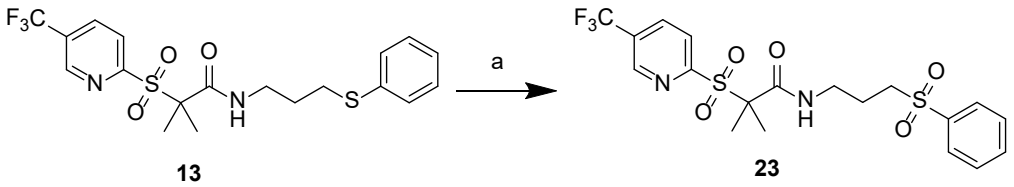
result for this reaction.

*Table 2 Condition Optimization of N-methylation Reaction for Compound **22***

base	solvent	yield %
K_2CO_3	Water/Dioxane	trace
TEA	DCM	trace
TEA	DMF	13%
NaH	DMF	41%

23

*Scheme 13 Reaction Scheme to Compound **23***



Reagents and conditions[49]: (a) OXONE, room temperature, overnight

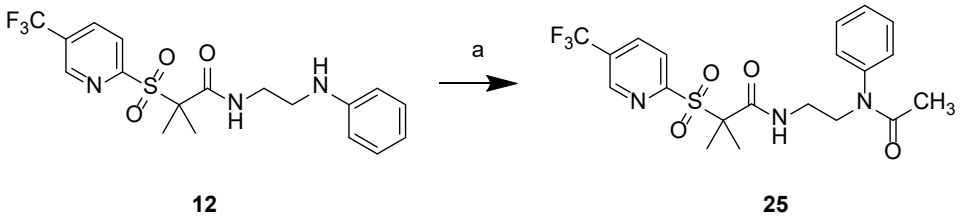
By using OXONE, the sulfone derivative can be obtained. Different from **Scheme 8**, 2 equivalents of OXONE were enough for this reaction, the reason may be a less steric hindrance on this S atom compared to the S atom next to the pyridine ring. The solvent using in this reaction was the same as **Scheme 8**. However, a methanol and water mixture can also achieve fair yield as shown in **Table 3**.

*Table 3 Solvent Condition Optimization of Oxidation Reaction for Compound **23***

solvent	yield %
Methanol/Water	33%
Dioxane/Water	45%

25

*Scheme 14 Reaction Scheme to Compound **25***



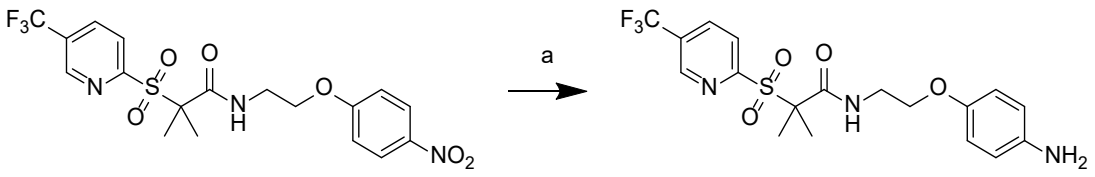
12 **25**

Reagents and conditions: (a) formyl chloride, Et_3N, DCM, room temperature, overnight

The acylation reaction was performed showing in **Scheme 14**. The 1.2 equivalents of Et_3N was used in this reaction serving as a base. Then, the nitrogen with a negative charge attacked the acetyl chloride to form compound **25**. The selectivity of the specific N atom comparing to another N atom on amide bond was relatively good. The reason is that this N atom is next to the aromatic ring, which makes this N atom more easily to react compared to the N atom on the amide bond. The total reaction yield was 70%.

31

*Scheme 15 Reaction Scheme to Compound **31***



18 **31**

Reagents and conditions: (a) H_2, Pd/C, THF/MeOH, 72 h.

The reduction reaction was achieved under catalytic hydrogenation condition as shown in **Scheme 15**. Several solvents were tested in this reaction. Like shown in

Table 4, a mixed one is better for both solubility and reaction rate. Using a mixture of THF/Methanol as the solvent can achieve a good yield of the final product. The Pd/C catalyze was selected to finish this reduction reaction and achieved a fair yield. Moreover, keep H_2 inside the reaction flask was a critical step to make this reduction reaction happening.

*Table 4 Solvent Condition Optimization of Reduction Reaction for Compound **31***

solvent	yield %
DCM	8%
THF	11%
MeOH	Trace
THF/MeOH	26%

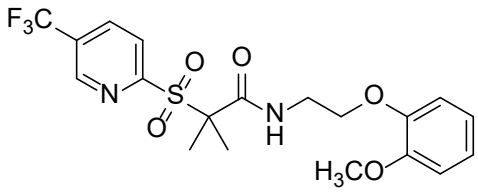
Synthesized Compounds Characterization

General

Those compounds listed below were prepared following the procedure described in Chapter 2 Method part. Some specific compounds synthetic routes (compound **22**, compound **23**, compound **25**, compound **31**) were also discussed in Chapter 3 Results and Discussion part.

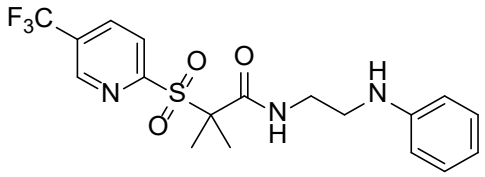
All of the compounds listed below possess ^{1}H NMR data to confirm their structure while some of them were described in ^{13}C NMR and high-resolution mass spectrometry (HRMS) due to the time issue and purity problems. Purity tests were performed in most of the compounds. Some published compounds have been cited.

11



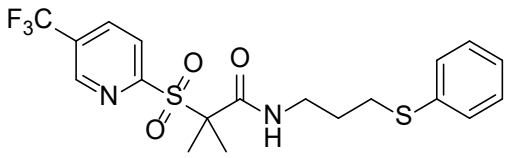
N-(2-(2-methoxyphenoxy)ethyl)-2-methyl-2-((5-(trifluoromethyl)pyridin-2-yl)sulfonyl)propanamide (**11**). Yellow solid (70 mg, 62%), Purified using EtOAc:Hexane 0-70% in 20 minutes; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.85 (s, 1H), 8.19 (d, *J* = 8.2 Hz, 1H), 7.89 (dd, J_1 = 8.2 Hz, J_2 = 1.9 Hz, 1H), 7.54 (t, *J* = 5.2 Hz, 1H), 6.99-6.96 (m, 1H), 6.92-6.89 (m, 3H), 4.05 (t, *J* = 5.1 Hz, 2H), 3.86 (s, 3H), 3.65 (dd, J_1 = 10.6 Hz, J_2 = 15.8 Hz, 2H), 1.70 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 167.74, 158.51, 150.05, 147.85, 147.04, 135.43 (q, *J* = 3.8 Hz), 130.12, 124.69, 122.39, 121.05, 115.06, 112.16, 101.23, 68.19, 68.02, 55.89, 40.00, 29.31, 20.49; HPLC purity (MeOH-Water gradient), 97.4%

12



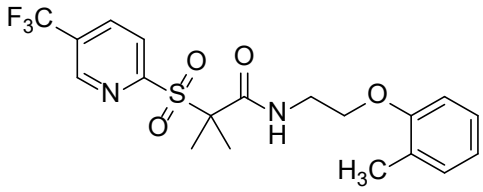
2-methyl-N-(2-(phenylamino)ethyl)-2-((5-(trifluoromethyl)pyridin-2-yl)sulfonyl)propenamide (**12**).[50] White solid (88 mg, 74%), Purified using EtOAc:Hexane 0-70% in 20 minutes; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.79 (s,1H), 7.93 (dd, J_1 = 8.4 Hz, J_2 = 2.4 Hz, 1H), 7.31 (d, *J* = 8.4 Hz, 1H), 7.16 (t, *J* = 7.5 Hz, 2H), 6.69 (t, *J* = 7.3 Hz, 1H), 6.57 (d, *J* = 7.9 Hz, 2H), 4.36 (s, 1H), 4.17-4.12 (m, 2H), 3.42 (t, *J* = 6.0 Hz, 2H), 2.71 (m, 1H), 1.14 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 168.70, 147.38, 143.88, 135.82, 135.64 (q, *J* = 5.6 Hz), 130.38, 129.31, 129.90, 125.35, 124.53, 123.11, 69.19, 68.31, 52.78, 36.29, 30.93, 20.37, 20.02; HPLC purity (MeOH-Water gradient), 97.6%

13



2-methyl-N-(3-(phenylthio)propyl)-2-((5-(trifluoromethyl)pyridin-2-yl)sulfonyl)propenamide (**13**).[50] White solid (102mg, 86%), Purified using EtOAc:Hexane 0-70% in 20 minutes; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.92 (s, 1H), 8.17 (s, 2H), 7.35 (s, 1H), 7.33 (s, 1H), 7.28-7.25 (m, 2H), 7.18-7.16 (m, 1H), 7.11 (t, *J* = 5.4Hz, 1H), 3.44 (dd, J_1 = 6.4 Hz, J_2 = 3.7 Hz, 2H), 3.03 (t, *J* = 7.2 Hz, 2H), 1.92 (m, *J* = 7.0 Hz, 2H), 1.62 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 167.51, 147.27, 135.94, 135.50 (q, *J* = 7.1 Hz), 131.23, 129.43, 129.36, 129.01, 126.21, 124.69, 124.44, 124.12, 67.56, 54.18, 39.31, 39.11, 31.10, 28.50, 20.50; HPLC purity (MeOH-Water gradient), 95.3%

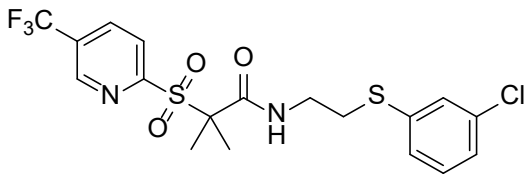
14



2-methyl-N-(2-(o-tolyloxy)ethyl)-2-((5-(trifluoromethyl)pyridin-2-yl)sulfonyl)propenamide (**14**). Yellow solid (65 mg, 88%), Purified using EtOAc:Hexane 0-70% in 20 minutes; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.66 (d, *J* = 0.7 Hz, 1H), 8.06 (d, *J* = 8.2 Hz, 1H), 7.81 (dd, J_1 = 2.0 Hz, J_2 = 8.2 Hz, 1H), 7.80 (t, *J* = 5.5 Hz, 1H), 7.16 (q, *J* = 7.8 Hz, 1H), 7.11 (d, *J* = 7.2 Hz, 1H), 6.88 (t, *J* = 7.4Hz, 1H), 6.77 (d, *J* = 8.1 Hz, 1H), 4.03 (t, *J* = 5.0 Hz, 2H), 3.72 (dd, J_1 = 5.2 Hz, J_2 = 15.8 Hz, 2H), 2.22 (s, 3H), 1.68 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 167.85, 158.20, 158.49, 147.11, 147.08, 135.45 (q, *J* = 4.5 Hz), 126.87, 126.84, 124.18, 123.37,

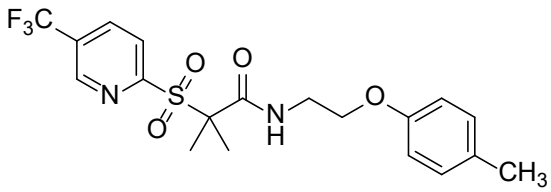
121.20, 120.97, 110.59, 67.66, 65.97, 40.09, 20.57, 16.18; HPLC purity (MeOH-Water gradient), 98.2%

15



N-(2-((3-chlorophenyl)thio)ethyl)-2-methyl-2-((5-(trifluoromethyl)pyridin-2-yl)sulfonyl)propenamide (**15**).[50] White solid (110 mg, 89%), Purified using EtOAc:Hexane 0-70% in 20 minutes; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.93 (s, 1H), 8.23-8.18 (m, 2H), 7.40 (t, *J* = 5.4 Hz, 1H), 7.37 (t, *J* = 1.8 Hz, 1H), 7.28-7.17 (m, 3H), 3.53 (dd, J_1 = 6.4 Hz, J_2 = 3.7 Hz, 2H), 3.15 (t, *J* = 6.6 Hz, 2H), 1.64 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 167.81, 158.12, 147.20, 147.17, 137.23, 135.57(q, *J* = 3.5 Hz), 134.89, 130.45, 130.16, 129.12, 127.48, 126.65, 124.43, 67.58, 39.48, 32.66, 20.44; HPLC purity (MeOH-Water gradient), 95.0%

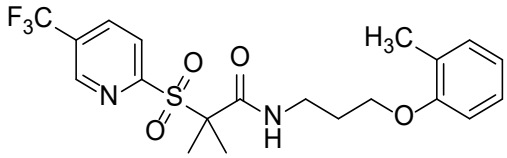
16



2-methyl-N-(2-(p-tolyloxy)ethyl)-2-((5-(trifluoromethyl)pyridin-2-yl)sulfonyl)propenamide (**16**). White solid (84 mg, 74%), Purified using EtOAc:Hexane 0-70% in 20 minutes; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.75 (s, 1H), 8.13 (d, *J* = 8.1 Hz, 1H), 7.80 (dd, J_1 = 1.4 Hz, J_2 = 8.2 Hz, 1H), 7.54 (t, *J* = 5.2 Hz, 1H), 7.10 (d, *J* = 8.4 Hz, 2H), 6.80 (d, *J* = 8.5 Hz, 2H), 4.00 (q, *J* = 5.0 Hz, 2H), 3.68 (dd, J_1 = 5.2 Hz, J_2 = 15.8 Hz, 2H), 2.30 (s, 3H), 1.71 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 167.83, 158.18, 156.34, 147.06, 147.03, 135.45 (q, *J* = 6.0 Hz), 130.68, 130.17,

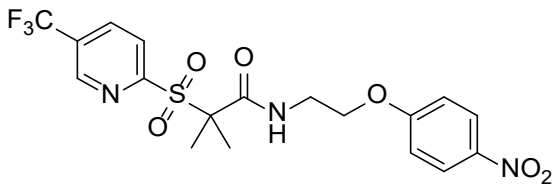
130.06, 124.35, 122.85, 121.15, 114.36, 67.83, 66.19, 40.04, 20.56, 20.40; HPLC purity (MeOH-Water gradient), 95.3%

17



2-methyl-N-(3-(o-tolyloxy)propyl)-2-((5-(trifluoromethyl)pyridin-2-yl)sulfonyl)propenamide (**17**). White solid (68 mg, 63%), Purified using EtOAc:Hexane 0-70% in 20 minutes; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.89 (s, 1H), 8.18-8.13 (m, 2H), 7.18-7.13 (m, 3H, include the NH s peak), 6.90-6.83 (m, 2H), 4.10 (t, *J* = 6.0 Hz, 2H), 3.56 (q, *J* = 6.4 Hz, 2H), 2.27 (s, 3H), 2.13 (p, *J* = 12.7 Hz, 2H), 1.66 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 167.52, 158.12, 147.19, 147.16, 135.50, 135.45 (q, *J* = 4.3 Hz), 130.35, 130.08, 126.82, 126.79, 124.53, 120.61, 111.05, 102.12, 67.75, 65.29, 37.76, 28.88, 20.49, 16.24; HPLC purity (MeOH-Water gradient), 97.3%

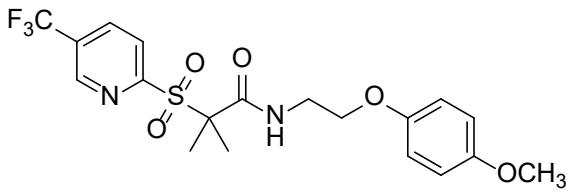
18



2-methyl-N-(2-(4-nitrophenoxy)ethyl)-2-((5-(trifluoromethyl)pyridin-2-yl)sulfonyl)propenamide (**18**). Yellow solid (90mg, 70%), Purified using EtOAc:Hexane 0-70% in 20 minutes; ^{1}H NMR(500 MHz, $CDCl_3$) δ: 8.76 (s, 1H), 8.17 (d, *J* = 9.2 Hz, 2H), 8.01 (dd, J_1 = 2.2Hz, J_2 = 8.4 Hz, 1H), 7.54 (d, *J* = 8.4 Hz, 1H), 6.86 (d, *J* = 9.2 Hz, 2H), 4.38-4.32 (m, 4H), 2.84 (p, *J* = 6.7 Hz, 1H), 1.16 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 178.41, 163.24, 158.53, 146.07, 141.82,

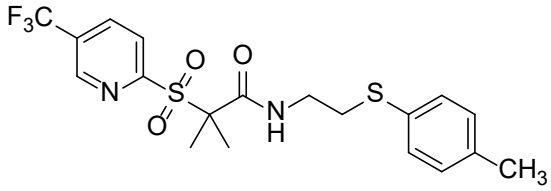
135.44 (q, *J* = 4.8 Hz), 130.55, 125.96, 124.34, 122.18, 120.45, 117.51, 114.36, 66.90, 47.25, 35.53, 29.70, 19.67; HPLC purity (MeOH-Water gradient), 97.4%

19



N-(2-(4-methoxyphenoxy)ethyl)-2-methyl-2-((5-(trifluoromethyl)pyridin-2-yl)sulfonyl)propenamide (**19**). White solid (48 mg, 73%), Purified using EtOAc:Hexane 0-70% in 20 minutes; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.76 (s, 1H), 8.11 (d, *J* = 8.2 Hz, 1H), 7.86 (dd, J_1 = 8.2 Hz, J_2 = 9.9 Hz, 1H), 7.59 (t, *J* = 5.5 Hz, 1H), 6.81 (t, *J* = 9.5 Hz, 4H), 3.95 (t, *J* = 5.1 Hz, 2H), 3.73 (s, 3H), 3.64 (dd, J_1 = 5.2 Hz, J_2 = 15.8 Hz, 2H), 1.67 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 167.80, 158.21, 154.29, 152.56, 147.06, 147.03, 135.45 (q, *J* = 6.2 Hz), 130.18, 129.92, 124.37, 121.24, 115.51, 114.77, 67.83, 66.80, 55.70, 40.04, 20.56; HPLC purity (MeOH-Water gradient), 95.3%

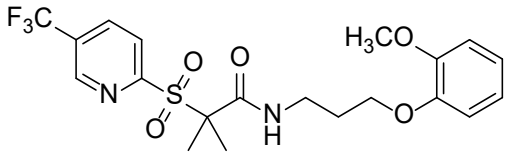
20



2-methyl-N-(2-(p-tolylthio)ethyl)-2-((5-(trifluoromethyl)pyridin-2-yl)sulfonyl)propenamide (**20**). White solid (80 mg, 76%), Purified using EtOAc:Hexane 0-70% in 20 minutes; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.91(d, *J* = 0.5 Hz, 1H), 8.20 (d, *J* = 8.2 Hz, 1H), 8.14 (dd, J_1 = 8.2 Hz, J_2 = 1.8 Hz, 1H), 7.35 (t, *J* = 5.2 Hz, 1H), 7.32 (s, 1H), 7.30 (s, 1H), 7.12 (s, 1H), 7.11 (s, 1H), 3.50 (dd, J_1 = 6.3 Hz, J_2 = 6.0 Hz, 2H), 3.06 (m, 2H), 2.32 (s, 3H), 1.64 (s, 6H); ^{13}C NMR (125 MHz,

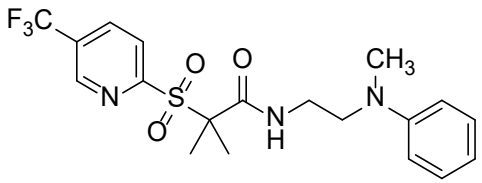
$CDCl_3$) δ: 167.62, 158.20, 147.17, 147.14, 137.04, 135.49 (q, *J* = 6.8 Hz), 130.93, 130.37, 130.22, 129.95, 124.48, 124.10, 123.48, 67.74, 39.46, 33.68, 21.00, 20.45; HPLC purity (MeOH-Water gradient), 95.2%

21



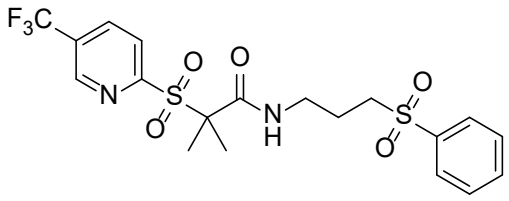
N-(3-(2-methoxyphenoxy)propyl)-2-methyl-2-((5-(trifluoromethyl)pyridin-2-yl)sulfonyl)propenamide (**21**). White solid (60 mg, 83%), Purified using EtOAc:Hexane 0-70% in 20 minutes; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.90 (s, 1H), 8.17-8.13 (m, 2H), 7.30-7.28 (m, 1H), 6.95-6.85 (m, 4H, include the NH s peak), 4.11 (t, *J* = 5.99 Hz, 2H), 3.86 (s, 3H), 3.50 (q, *J* = 5.85 Hz, 2H), 2.06 (p, *J* = 6.2 Hz, 2H), 1.67 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 167.48, 158.46, 149.56, 148.10, 147.04, 135.33 (q, *J* = 3.4 Hz), 124.75, 121.67,120.87, 113.75, 111.79, 68.33, 67.79, 55.68, 38.44, 28.60, 20.32; HPLC purity (MeOH-Water gradient), 95.2%

22



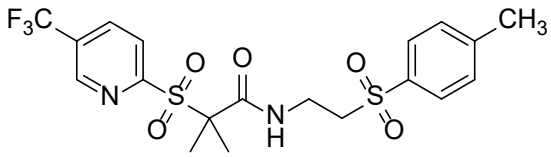
2-methyl-N-(2-(methyl(phenyl)amino)ethyl)-2-((5-(trifluoromethyl)pyridin-2-yl)sulfonyl)propenamide (**22**). White solid (21 mg, 86%), Purified using EtOAc:Hexane 0-80% in 20 minutes; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.73 (s, 1H), 7.83 (dd, J_1 = 8.4 Hz, J_2 = 1.6 Hz, 1H), 7.20 (t, *J* = 8.8 Hz, 2H), 7.18-7.13 (m, 2H, include the NH s peak), 6.67 (s, 3H), 4.09 (t, *J* = 6.7 Hz, 2H), 3.67 (t, *J* = 6.8 Hz, 2H), 2.83 (s, 3H), 1.12 (d, *J* = 6.7 Hz, 6H); HPLC purity (MeOH-Water gradient), 98.0%

23



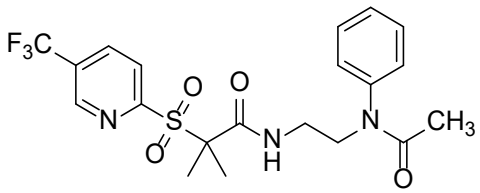
2-methyl-N-(3-(phenylsulfonyl)propyl)-2-((5-(trifluoromethyl)pyridin-2-yl)sulfonyl)propenamide (**23**). White solid (23 mg, 54%), Purified using EtOAc:Hexane 0-80% in 20 minutes; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.01 (s, 1H), 8.25-8.21 (m, 2H), 7.95-7.93 (m, 2H), 7.68 (t, *J* = 7.5 Hz, 1H), 7.58 (t, *J* = 7.9 Hz, 2H), 7.23 (t, *J* = 5.7 Hz, 1H), 3.49 (dd, J_1 = 6.3 Hz, J_2 = 6.4 Hz, 2H), 3.35 (t, *J* = 7.7 Hz, 2H), 2.10 (p, *J* = 6.8 Hz, 2H), 1.63 (s, 6H); HPLC purity (MeOH-Water gradient), 95.6%

24



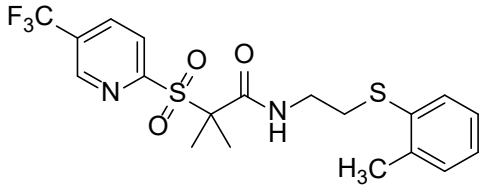
2-methyl-N-(2-tosylethyl)-2-((5-(trifluoromethyl)pyridin-2-yl)sulfonyl)propenamide (**24**). Yellow solid (30 mg, 45%), Purified using EtOAc:Hexane 0-80% in 20 minutes; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.91 (s, 1H), 8.23 (s, 1H), 7.83 (s, 1H), 7.81 (s, 1H), 7.58-7.56 (m, 1H), 7.29 (s, 1H), 7.38 (s, 1H), 3.70 (dd, J_1 = 6.1Hz, J_2 = 6.3 Hz, 2H), 3.37 (t, *J* = 6.2 Hz, 2H), 2.46 (s, 3H), 1.63(s, 6H)

25



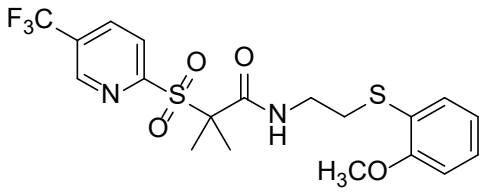
2-methyl-N-(2-(N-phenylacetamido)ethyl)-2-((5-(trifluoromethyl)pyridin-2-yl)sulfonyl)propenamide (**25**). White solid (24 mg, 70%), Purified using EtOAc:Hexane 0-70% in 20 minutes; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.96 (s, 1H), 8.20 (s, 1H), 8.20 (s, 1H), 7.57 (s, 1H), 7.45-7.42 (m, 2H), 7.39-7.36 (m, 1H), 7.28-7.26 (m, 2H), 3.95 (t, *J* = 5.6 Hz, 2H), 3.42 (dd, J_1 = 6.7 Hz, J_2 = 16.1 Hz, 2H), 2.46 (s, 3H), 1.63 (s, 6H), 1.65 (s, 6H); HPLC purity (MeOH-Water gradient), 97.4%

26



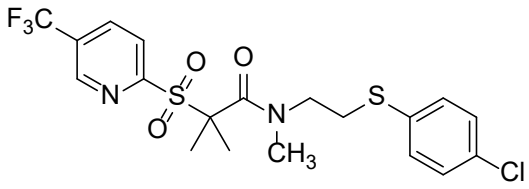
2-methyl-N-(2-(o-tolylthio)ethyl)-2-((5-(trifluoromethyl)pyridin-2-yl)sulfonyl)propenamide (**26**).[50] White solid (66 mg, 74%), Purified using EtOAc:Hexane 0-70% in 20 minutes; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.90 (s, 1H), 8.18 (d, *J* = 8.2 Hz, 1H), 8.12 (dd, J_1 = 8.2 Hz, J_2 = 1.8 Hz, 1H), 7.40-7.35 (m, 2H, include NH br peak), 7.19-7.11 (m, 3H), 3.52 (dd, J_1 = 6.3 Hz, J_2 = 6.1 Hz, 2H), 3.11 (t, *J* = 6.6 Hz, 2H), 2.39 (s, 3H); HPLC purity (MeOH-Water gradient), 95.2%

27



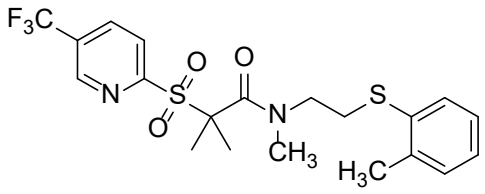
N-(2-((2-methoxyphenyl)thio)ethyl)-2-methyl-2-((5-(trifluoromethyl)pyridin-2-yl)sulfonyl)propenamide (**27**).[50] Yellow solid (80 mg, 71%), Purified using EtOAc:Hexane 0-80% in 20 minutes; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.96 (s, 1H), 8.23 (d, *J* = 8.2 Hz, 1H), 8.17 (dd, J_1 = 8.3 Hz, J_2 = 1.8 Hz, 1H), 7.44 (dd, J_1 = 7.6 Hz, J_2 = 1.6 Hz, 2H), 7.28 (s, 1H), 6.97-6.90 (m, 2H), 3.94 (s, 3H), 3.47 (dd, J_1 = 6.2 Hz, J_2 = 6.1 Hz, 2H), 3.07 (t, *J* = 6.4 Hz, 2H), 1.67 (s, 6H)

28



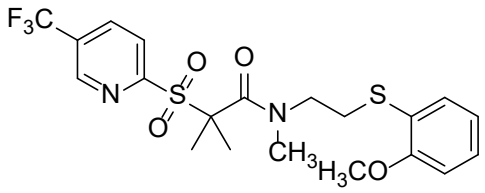
N-(2-((4-chlorophenyl)thio)ethyl)-N,2-dimethyl-2-((5-(trifluoromethyl)pyridin-2-yl)sulfonyl)propenamide (**28**). White solid (23 mg, 60%), Purified using EtOAc:Hexane 0-80% in 20 minutes; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.91 (s, 1H), 8.21-8.15 (m, 2H), 7.30-7.25 (m, 4H), 3.51 (s, 2H), 3.29-3.28 (m, 3H), 3.06 (t, *J* = 7.4 Hz, 2H), 1.82 (s, 6H); HPLC purity (MeOH-Water gradient), 95.8%

29



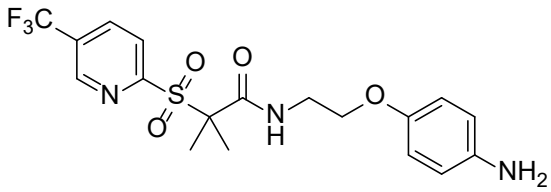
N,2-dimethyl-N-(2-(o-tolylthio)ethyl)-2-((5-(trifluoromethyl)pyridin-2-yl)sulfonyl)propenamide (**29**). White solid (30 mg, 54%), Purified using EtOAc:Hexane 0-80% in 20 minutes; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.91 (s, 1H), 8.20 (d, *J* = 8.2 Hz, 1H), 8.12 (d, *J* = 8.1 Hz, 1H), 7.32 (d, *J* = 6.9 Hz, 1H), 7.18-7.12 (m, 3H), 3.56 (s, 2H), 3.05 (t, *J* = 7.5 Hz, 2H), 2.37 (s, 3H), 1.80 (s, 6H); HPLC purity (MeOH-Water gradient), 96.5%

30



N-(2-((2-methoxyphenyl)thio)ethyl)-N,2-dimethyl-2-((5-(trifluoromethyl)pyridin-2-yl)sulfonyl)propenamide (**30**). Yellow solid (33 mg, 70%), Purified using EtOAc:Hexane 0-70% in 20 minutes; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.97 (s, 1H), 8.17 (d, *J* = 8.2 Hz, 1H), 8.12 (d, *J* = 8.2 Hz), 7.39 (d, *J* = 7.6 Hz, 1H), 7.19 (t, *J* = 7.5 Hz, 1H), 6.88-6.82 (m, 2H), 3.85 (s, 3H), 3.24 (t, *J* = 7.1 Hz, 2H), 3.11-3.07 (m, 2H), 2.65 (s, 3H), 1.61 (s, 6H)

31



N-(2-(4-aminophenoxy)ethyl)-2-methyl-2-((5-(trifluoromethyl)pyridin-2-yl)sulfonyl)propenamide (**31**). White solid (16 mg, 30%), Purified using MeOH:Dichloromethane 0-10% in 20 minutes; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.81 (d, *J* = 0.6 Hz, 1H), 8.13 (d, *J* = 8.2 Hz, 1H), 7.89 (dd, J_1 = 8.2 Hz, J_2 = 1.6 Hz, 1H), 7.46 (t, *J* = 5.1 Hz, 1H), 6.72 (m, *J* = 2.3 Hz, 4H), 3.94 (t, *J* = 5.1 Hz, 2H), 3.64 (dd, J_1 = 5.1 Hz, J_2 = 15.8 Hz, 2H), 1.69 (s, 6H)

Biological Evaluation Results

We studied the number and size of inclusions to determine the compounds' activity.[51, 52] The Immunofluorescence assay (IFA) was utilized to analyze the antichlamydial activity according to recent literature.[53, 54] The experimental details are shown in the Methods Chapter. Briefly, the human epithelial cell line HEp-2 was cultured and plated, then the *Chlamydia trachomatis serovar L2* was added to infect those cells. After 8 hours post-infection, the synthesized compounds were added to the plate, at 50 µg/mL in DMSO solution. After 16 hours, total 24 hours post-infection, we compared numbers and size of inclusions with control, untreated cells and the morphology and viability of the cells were examined. The qualitative results (primary infection; IFA result) are shown below in **Table 5**. Also, the standard images to differentiate effective group, intermediate effective group and not effective group is listed below in **Figure 6**.

Table 5 IFA Biological Evaluation Result of Synthesized Compounds

compound number	inhibition activity
11	-
13	+
14	-
15	+
16	++
17	++
18	-
19	-
20	+
21	-
22	-
23	+
25	-
26	-
28	+
29	-

Compounds were tested at 50 μg/mL. The ability of inhibition on Chlamydial inclusions of each compound falls into 3 categories: [++] = effective; [+] = intermediate effective; [-] = not effective. The control group is untreated cells or only DMSO added cells.

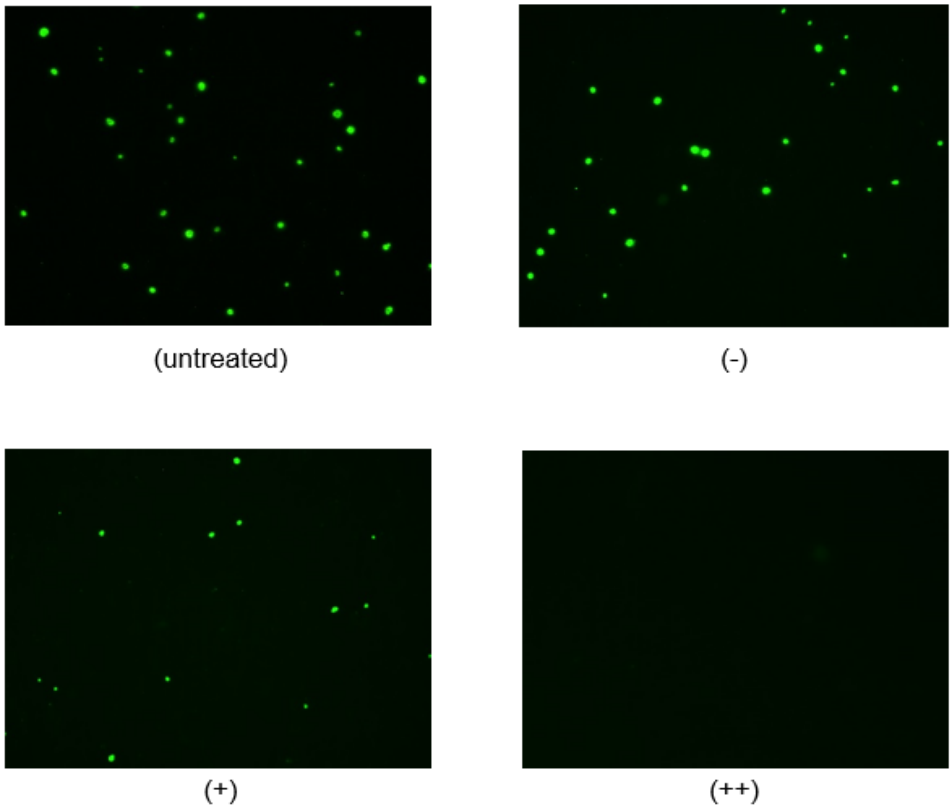


Figure 6 Immunofluorescence images of four groups with different anti-chlamydia activity

Four different groups: untreated group; (-) not effective group; (+) intermediate effective group; (++) effective group; IFA assay (50 μg/mL); in green, chlamydial inclusions.

CHAPTER 4 CONCLUSION

Chlamydia trachomatis infection is a severe problem affecting the general population, especially reproductive-age females. It has been proposed that the two caseinolytic protease paralogues, ClpP1 and ClpP2, may play essential roles in *Chlamydia trachomatis* growth.[43] In this study, 21 compounds were synthesized based on the structure of known ClpP activators to halt the growth of *Chlamydia*. The synthesized compounds were divided into five categories (**Figure 6**). Group A possesses an O atom in the right part of the molecule, group B has three carbon linker in the middle of the molecule, group C owns the N atom in the right part of the molecule. Group D is almost the same as standard molecule ACP1b and we changed the group on the right part of the molecule. We added one methyl group on the N atom on the amide bond in group E.

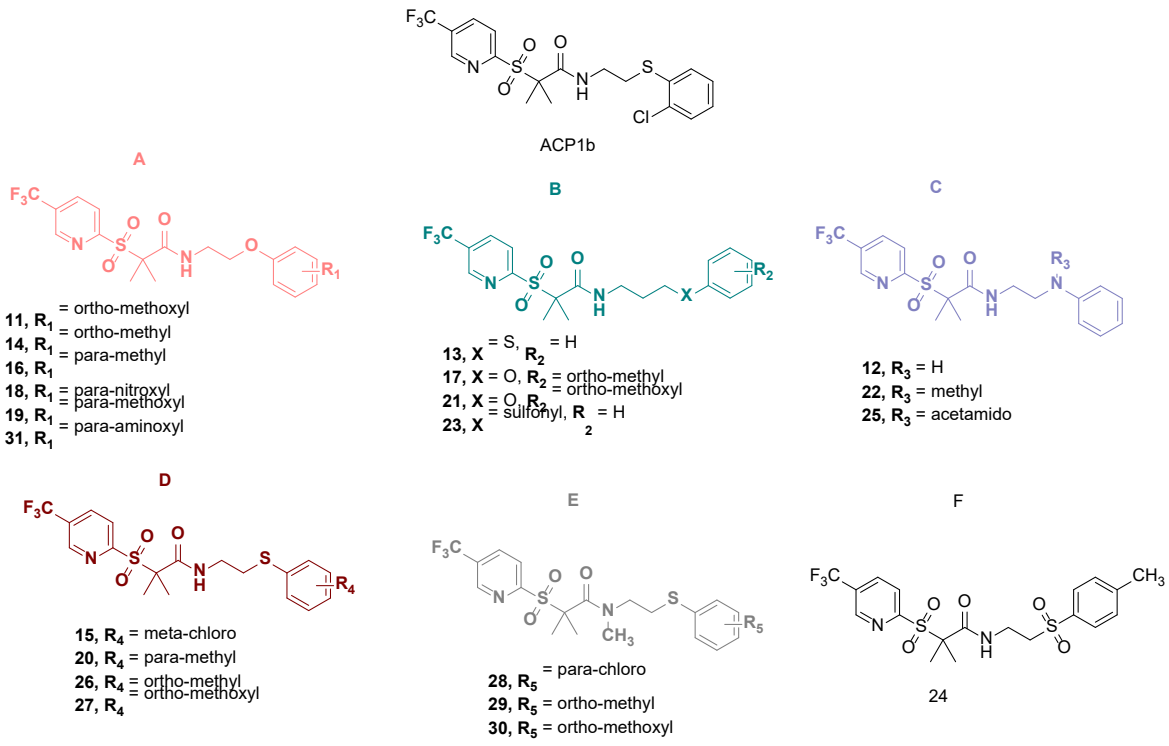


Figure 7 All synthesized compounds comparing with ACP1b.

We found that derivatives **11**, **14**, **18** and **19**, analogs with O atom in the right part of the molecule were not active at 50 μg/mL. Likewise, compounds **22** and **25** did not show any antichlamydial activity. However, several compounds like **13**, **15**, **20** and **28** performed relatively good activity. This may indicate that the S atom is a better choice than the O atom and N atom on the right part of the molecule. According to **Table 6**, it seems the compounds with higher LogP values (over 3.5) (calculated by ChemDraw) will have better activity although there are some exceptions. We hypothesis that the compounds with greater lipophilicity may have better antichlamydial activity. Those lipophilicity compounds may penetrate cell membrane more easily than other compounds which lead to greater anti-chlamydial activity.[55]

Table 6 LogP Value of the Synthesized Compounds

compounds No.	LogP	activity
11	2.83	-
13	3.63	+
14	3.45	-
15	4.09	+
16	3.45	++
17	3.55	++
18	3.93	-
19	2.83	-
20	4.01	+
21	2.94	-
22	3.57	-
23	2.07	+
25	2.23	-
26	4.01	-
28	4.32	+
29	4.25	-

It is highlighted that a longer carbon linker in the middle of the molecule may be good for the activity from the positive results of compounds **13**, **17** and **23**. All those three

molecules possess a 3-carbon linker in the middle of the molecule and they have better antichlamydial activity comparing to other tested compounds.

Also, we noticed that **16**, **17** and **20** with methyl group possessed relatively good activity while **11**, **19** and **21** with methoxy group did not have any activity. Compounds like **15** and **28** which have chlorine atoms in the right part of the molecule possessed intermediate activity. This should be noted that the molecule with a methyl group or Cl group in the right part of the molecule may have better antichlamydial activity.

Currently, we are working on making three carbon and four carbon linker analogs. Besides, different analogs that own heterocycles on the left part of the molecule are being prepared. The combining result suggests that ACP derivatives could be a promising leading structure specifically targeting *Chlamydia trachomatis* for further development.

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