Ligand Synthesis for Hepatitis C Virus Internal Ribosome Entry Site

Wang, Zihao

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Ligand Synthesis for Hepatitis C Virus Internal Ribosome Entry Site

A Thesis submitted in partial satisfaction of the requirements for the degree

Master of Science

in

Chemistry

by

Zihao Wang

Committee in charge:

Professor Thomas Hermann, Chair
Professor Simpson Joseph
Professor Emmanuel Theodorakis

2017
The Thesis of Zihao Wang is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Chair

University of California, San Diego

2017
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ABSTRACT OF THE THESIS

Ligand Synthesis for Hepatitis C Virus Internal Ribosome Entry Site

by

Zihao Wang

Master of Science in Chemistry

University of California, San Diego, 2017

Professor Thomas Hermann, Chair

Benzothiadiazines were synthesized and tested for their binding affinity with hepatitis C virus internal ribosome entry site subdomain IIa using FRET assay. The acetylated compound was designed to improve the solubility and explore the tautomerization involved in targeting the IIa subdomain. An optimized
Cu₂O catalyzed Ullmann reaction was developed for future use.
Introduction
1.1 Hepatitis C Virus

HCV is a flavivirus responsible for non-A and non-B hepatitis. HCV has been regarded as a major cause of severe liver damage since people with chronic infection are likely to develop cirrhosis, hepatic steatosis, and, occasionally, Hepatocellular carcinoma – the third leading cause of cancer deaths in the world. HCV is predominantly acquired through contaminated needles and improperly-screened blood transfusions. People with acute HCV infection are usually asymptomatic, if left undiagnosed and untreated they could easily transform into chronic HCV and cirrhosis. With an estimated number of 71 million people who are diagnosed with chronic hepatitis C infection, and 399,000 death caused by hepatitis C-related liver diseases each year, HCV remains to be a major global health issue. Since the first identification of HCV in 1989, HCV has displayed a high degree of genetic diversity with 7 genotypes comprising 67 subtypes recognized, making the development of HCV vaccines and pan-genotypic therapies quite challenging.
1.2 The evolution of approved treatment regimens against HCV.

The goal for HCV treatment is to achieve a sustained virologic response (SVR), and patients maintaining a SVR for 24 weeks after the end of treatment are considered a ‘cure’. Initially, patients with HCV were treated with interferon-α solely for an efficacy of less than 10%. Later on, several treatments based on interferon-α were developed. The first treatment approved by FDA, which is interferon-α in combination with ribavirin, has a cure rate of about 34-42%. Prior to the introduction of DAAs, pegylated interferon-α associated with ribavirin reached a cure rate of about 50%. In 2015, FDA approved a new treatment regimen containing ombitasvir (PTV/r/OBV) with dasabuvir, since then, the cure rate for HCV especially genotype 1 has been significantly improved. The direct acting antivirals (DAAs) are groups of compounds that could inhibit the replication process of HCV by directly acting on the viral proteins. The cure rates for HCV genotype I could reach to nearly 100% according to several patient groups. Developed by using high-throughput screening, DAAs have been assigned into
four classes (Table 1.2.1). However, the highly efficacious regimen is not accessible to the majority of the patients in need due to its expensive price. The DAAs are not available to up to 59 million patients throughout the world with a price of about 89,760 USD for each treatment in the United States, and the drug does not exist in the market of Argentina, Brazil, China, Russia, and Ukraine.\textsuperscript{13,14}

Table 1.2.1. (A) Currently approved direct-acting antivirals (DAAs) based on the work form T Chen et al;\textsuperscript{15} (B) Wholesale acquisition cost (WAC) of some pangenotypic DAAs, adapted from the results at E Rosenthal's group.\textsuperscript{16}

<table>
<thead>
<tr>
<th>A</th>
<th>Category</th>
<th>Drugs Available</th>
</tr>
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<tbody>
<tr>
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<td>NS3/4A protease inhibitors</td>
<td>paritaprevir/ritonavir; simprevir; asunaprevir; grazoprevir</td>
</tr>
<tr>
<td></td>
<td>NS5A replication complex inhibitors</td>
<td>ombitasvir; daclatasvir; ledipasvir; elbasvir; velpatasvir</td>
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<tr>
<td></td>
<td>Nucleotide NS5B polymerase inhibitors</td>
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<tr>
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<td>Nonnucleoside NS5B polymerase inhibitors</td>
<td>dasabuvir</td>
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<tr>
<td></td>
<td>Viekira Pak</td>
<td>ombitasvir, paritaprevir, ritonavir, and dasabuvir</td>
<td>AbbVie Inc.</td>
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<tr>
<td></td>
<td>Zepatier</td>
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<td>Merck Sharp Dohme</td>
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</table>
1.3 The epidemiology of HCV

Among all of the HCV genotypes, genotype 1 is considered to account for the most HCV cases worldwide at about 83.4 million (46.2%). However, the diversity of HCV genotypes varies by area. In China and many countries in Southeast Asia, Western Europe and Australia, the diversity of HCV genotypes is high. And the relative genotype frequencies for each Global Burden of Disease (GBD) region are different. According to the survey by Messina et al, genotype 2 dominates West Africa, genotype 3 is the most frequent in south Asia and parts of Scandinavia, genotype 4 prevails in Central and North Africa, genotype 5 has the highest incident rate in South Africa, and genotype 5 is prevalent in Southeast Asia (Figure 1.3.1). Thus, developing pan-genotypic antivirals is necessary in combating HCV. While the current DAAs are effective in treating HCV genotypes 1 & 2, there is not enough data regarding the remaining genotypes. Due to the high mutation rate of the RNA virus, antiviral resistance could be a potential threat for future treatment. If the relatively less frequent genotypes could not be
controlled, they are likely to be developed into the next major infections worldwide.\textsuperscript{14}

Figure 1.3.1. Relative prevalence of each HCV genotype by Global Burden of Disease (GBD) Regions. Figure modified from J P. Messina et al.\textsuperscript{17}

1.4 The internal ribosomal entry site of HCV

The replication of HCV starts by using its single-stranded RNA genome as the messenger RNA to produce the viral proteins. The 5’ untranslated region (UTR) of the HCV genome is highly conserved among different viral strains since it contains a large structured domain that serves as an internal ribosomal entry
site (IRES).\textsuperscript{18} The IRES resided on the 5'-UTR is crucial in facilitating the initiation of HCV protein translation, and because the required IRES-mediated translation is distinct from how most cellular proteins are made, the HCV IRES has been regarded as an attractive target for developing novel anti-HCV therapeutics.\textsuperscript{19} The IRES directly regulates the assembly and functions of translation initiation complexes by recruiting the host cell 40S ribosomal subunit via a 5' cap-independent mechanism.\textsuperscript{20}

![Figure 1.4.1. Cap-independent translation initiation mechanism used by hepatitis C virus. (2 and 3 stands for eukaryotic initiation factor (eIF) 2 and eIF3, respectively.) Adapted from Davis et al.\textsuperscript{18}](image-url)
The IRES RNA actively manipulates the host cell’s 40S ribosomal subunit to organize the assembly of the pre-initiation complex through a conformational change in the subunit induced by the binding of IRES. As is shown in the structure complexes (figure 1.4.2) acquired by cryo-electron microscopy (cryo-EM) in combination with single particle approach. Several changes were observed on the 40S subunit after binding with IRES, including the orientation of the head relative to the body, the shape of the platform domain, as well as regions marked with asterisks, leading to the close of the mRNA binding cleft. The bent conformation of IIa region of IRES is crucial in clamping the HCV RNA onto the 40S unit. Domain II places and holds the single-stranded coding part of the RNA into the decoding center until the rest of the translation machinery has assembled and the elongation has started. The L-shaped conformation of the domain II is amenable for a correct binding of the viral mRNA at the ribosome. A cryo-EM reconstruction of the ribosome-bound HCV IRES with a resolution of 3.9 Å done by Nick Quade et al. further elucidated the interactions between the two (Figure
1.4.3). A detailed view of the contact area between the flexible domain II of the IRES and the 40S ribosomal subunit revealed that the domain II binds to the intersubunit side of the 40S at two sites. The apical loop of domain II interact with uS7 protein at E-site secures proper positioning of the mRNA in the mRNA channel, at the same time, the minor groove of domain II contacting with the eS25 protein is responsible for translation initiation as well.²²
Figure 1.4.2. Surface representation of the vacant 40S ribosomal subunit from rabbit reticulocytes (A to C), and the 40S-IRES complex (D to F). Some indications of the landmarks of the 40S subunit are: b, body; bk, beak; h, head; pt, platform; sh, shoulder.21
Within the HCV IRES, the highly conserved domain II contains a subdomain IIa, which is shown as an internal loop in the lower stem of the domain. (Figure 1.4.4) Previous research from our lab has found out that a benzimidazole 1 (Figure 1.4.5) could act as a HCV-specific translation inhibitor to induce a widened RNA interhelical angle at the IRES subdomain IIa, resulting an extended conformation. This lead to the prevention of the IRES function as well as the translation of the viral RNA due to an incorrect orientation of the apical hairpin
loop IIb at the ribosome. \( ^{23} \)

Figure 1.4.4. Secondary structure of the HCV 5' UTR. \( ^{24} \)

Figure 1.4.5. Benzimidazole inhibitors targeting HCV IIa subdomain.
1.5 FRET assay to determine the conformational change of IRES IIa

We discovered the conformational switch of the subdomain IIa in the presence of benzimidazole through fluorescence resonance energy transfer (FRET) experiments. The fluorescent cyanine dyes labeled subdomain IIa does not project any signals initially due to its flexibility to switch between bent and extended conformations exceeds the Forster radius. However, when titrated with Mg$^{2+}$, FRET signals arise, indicating a folded RNA-Mg$^{2+}$ complex formed.

Subsequently a titration was performed on subdomain IIa RNA with benzimidazole in the presence of 2mM Mg$^{2+}$. A dose-dependent quenching of FRET by adding benzimidazole suggested that ligand binding at the internal loop induced a widening of the interhelical angle at the IRES subdomain IIa (Figure 1.5.1).

The flexibility of the IIa subdomain is indispensable to translation initiation. While the bent conformation ensures the docking of the subdomain IIb into the mRNA exit site on the 40S subunit, the widening of the angle at the internal loop
leads to the undocking of the IIb and terminates the IRES-driven translation.\textsuperscript{23}

Figure 1.5.1. FRET signal of Cy3/Cy5-labeled IIa RNA titrated with benzimidazole\textsubscript{1} under 2mM Mg\textsuperscript{2+}. The EC\textsubscript{50} value for benzimidazole\textsubscript{1} binding is 600\(\pm\)80nM.

The ligand-responsive conformational switch motif in the HCV domain II contains a binding site for guanosine that possibly acts as the cognate biological ligand. The weak binding affinity between guanosine ligand and the IIa target results in a transiently extended state of the switch and allows the assembled 80S ribosome undocking from the IRES.\textsuperscript{25}
Recent studies from Hermann’s lab has found out that the HCV-like IRES elements containing analogous domain II elements has been found in several other viruses belonging to the flavirius and picornavirus families, indicating potential targets for the discovery of viral translation inhibitors for viruses in these two families.

1.6 Binding pocket in HCV IRES subdomain IIa

By describing the co-crystal structure of subdomain IIa in complex with benzimidazole inhibitor, we have previously illustrated the ligand-binding pocket in subdomain IIa capable of binding with guanine and benzimidazole derivatives of similar structure.

The crystal structure acquired by X-ray crystallography determined that the IRES subdomain IIa to be an overall bent structure and this L-shaped structure is required for a correct binding of the viral mRNA at the ribosome (Figure 1.6.1A). When docked with a ligand 2, the helices flanking the internal loop were coaxially
stacked, transforming the RNA in the complex into a linear conformation (Figure 1.6.1B). The RNA internal loop refolded to form a pocket that deeply encapsulates the ligand2.

![Figure 1.6.1](image)

Figure 1.6.1. (A) Crystal structure of the IRES subdomain IIa stabilized by Mg$^{2+}$ ions (green spheres). (B) Crystal structure of the subdomain IIa RNA inhibitor complex (the benzimidazole ligand is in yellow). (C) Schematic of the interactions in the ligand-binding site (the dashed lines represent hydrogen bonds, and stacked lines indicate stacking of bases and intercalation of the benzimidazole2. The red highlighted bases are residues interacting with the ligands.).

When docked with benzimidazole2 (figure 1.4.5), the roof of the cavity is delineated by the C58-G100 base pair bonding with the ligand through the guanine Hoogsteen edge, and the floor is limited by the stacking interactions
between A53 and the G52-C111 base pair (Figure 1.6.1 B & C).

1.7 The optimization for ligands targeting IIa subdomain

After the discovery of 2-amino-benzimidazole 1 through mass-spectrometry screening, the ligand optimization led to the appearance of compound 2. The structure-activity relationships study of analogs derived from 1 indicated that substitutions at positions 6 and 7 would not be tolerated by the binding pocket, and the co-crystal structure of the benzimidazole 2 binding with the subdomain IIa RNA further validated the conclusion. The benzene ring of the 2-amino quinazolinone 3 (Figure 1.7.1) contributes to an improved stacking interactions with RNA bases G52 and A53, which resulted in a 2-fold better binding affinity compared to guanine.
To explore the binding pocket for better insights on ligand designing, we manually docked compound 3 and 4 to the crystal structure of the subdomain IIa RNA in complex with 2, through which a small pocket was emerged extending at the back side of the inhibitor target site (Figure 1.7.2). By occupying the back side of the pocket with the 7-methane group of 2-amino benzimidazoles, the carbonyl group of 2-amino-pyrimidinone derivatives (guanine and 3), or the spiro-cyclopropyl group of the compound 4, the ligands could position themselves correctly to bind with the subdomain IIa. It is conceivable that the back-side pocket could accommodate a small non-planar substituent.
Figure 1.7.2. A. The co-crystal structure of the 2-amino-benzimidazole (yellow) inhibitor and IIa subdomain complex. B. Guanine (green) and 2-amino-quinazolinone 3 (light blue) were manually docked to the HCV subdomain IIa RNA, revealing a back-side pocket. (The arrow indicates the pocket at the back side of the inhibitor target site)
Results and Discussion
2.1 The synthesis for acetylated benzothiadiazine derivatives

A previous Postdoc Gloria Hernández-Torres from our lab has found out that compounds with a scaffold of benzothiadiazine tend to have high binding affinities with HCV subdomain IIa RNA (Table 2.1.1). However, the compound has a poor solubility. To enhance the solubility of the compounds derived from this scaffold, we decided to add an acetyl group to the structure. At the same time, we would like to further explore the mechanism of action for the inhibitors binding with the IIa subdomain, expecting to achieve better binding affinities for novel antiviral agents.
Table 2.1.1 EC$_{50}$ value of compound 5 and 6 obtained from HCV IRES FRET assay.

<table>
<thead>
<tr>
<th>Structure</th>
<th>EC$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image.png" alt="Structure 5" /></td>
<td>11 ± 7 µM</td>
</tr>
<tr>
<td><img src="image.png" alt="Structure 6" /></td>
<td>47 ± 30 µM</td>
</tr>
</tbody>
</table>

2.1.1 Acetylation for the 3-amino-4-((3-(dimethylamino)propyl)amino)-1,2,4-benzothiadiazine 1,1-dioxide (5)
Scheme 2.1.1.1. Synthesis of benzothiadiazine compound 5

The starting material was previously synthesized by Walter Frauman through melting the 2-((3-(dimethylamino)propyl)amino) benzenesulfonamide and guanidine carbonate together under 200 °C (Scheme 2.1.1.1).28

Scheme 2.1.1.2. Synthesis of the acetylated benzothiadiazine 7

a: acetyl chloride (1.5 equivalent), triethylamine (3 equivalent), dichloromethane, room temperature, 12 hours.
The cyclized product was then treated with acetyl chloride and triethylamine for 12 hours under room temperature to afford the acetylated compound \(7\) (Scheme 2.1.1.2).\(^{29}\)

2.1.2 Acetylation for 3-amino-2,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide

The same procedure was applied to the compound \(6\) to obtain the acetylated compound \(8\) (Scheme 2.1.2.1).

![Reaction Scheme](attachment:image.png)

Scheme 2.1.2.1. Synthesis of compound \(8\) using the same procedure as in 2.1.1

2.1.3 Materials and Methods

Compound \(5\) (1 mmol, 281.4 mg) was dissolved in 10 mL of dichloromethane with magnetic stirring, triethylamine (3 mmol, 0.4 mL) and
acetylchloride (1.5 mmol, 0.107 mL) was added subsequently to the solution. 12 hours later, the solvent was removed in vacuo, ethyl acetate and water was added for extraction, the aqueous layer was further extracted with dichloromethane, and the organic layer was removed under reduced pressure to afford the yellow oil 7.

7: $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$(ppm): 7.760 (d, 1H, J=7.73Hz), 7.671 (t, 1H, J=7.78Hz), 7.590 (d, 1H, J=8.02Hz), 7.410 (t, 1H, J=7.39Hz), 4.094 (t, 2H, J=6.06Hz), 2.495 (t, 2H, J=6.46Hz), 2.444 (s, 6H), 2.325 (s, 3H), 2.158 (s, 1H), 1.899 (m, 2H); $^{13}$C NMR (500 MHz, DMSO-$d_6$) $\delta$(ppm): 172.554, 152.287, 137.215, 133.344, 125.698, 123.536, 118.289, 117.025, 54.181, 45.679, 43.426, 42.967, 24.882, 21.521; MS mass calculated $C_{14}H_{20}N_{4}O_{3}S$ [M+H]$^+$ = 325.13, found 325.47.

The same procedure was applied to the benzothiadiazine 6 to give the pure product 8 as white solid.
8: $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$(ppm): 7.798 (d, 1H, $J$=6.89 Hz), 7.668 (t, 1H, $J$=7.03 Hz), 7.582 (d, 1H, $J$=7.47 Hz), 7.446 (t, 1H, $J$=6.94 Hz), 2.178 (s, 3H); $^{13}$C NMR (500 MHz, DMSO-$d_6$) $\delta$(ppm): 174.268, 147.876, 134.023, 133.491, 126.749, 123.488, 123.197, 119.244, 24.456; HRMS mass calculated C$_9$H$_9$N$_3$O$_3$S $[M+Na]^+$ = 262.0257, found 262.0254, delta -1.1 ppm.
2.1.4 Spectral Data

Mass Spectrometry

Spectrum 2.1.4.1. 3-acetamide-4-((3-(dimethylamino)propyl)amino) 1,2,4-benzothiadiazine 1,1-dioxide (7) mass spectrometry.

ZW-66DD-a #48-53 RT: 0.51-0.56 AV: 6 SB: 4 0.42-0.45 NL: 1.32E7
T: + c Full ms (120.00-600.00)

[M+H]^+  ESI-MS Positive Ion Mode
Spectrum 2.1.4.2. 3-acetamide-2,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide (8) high resolution mass spectrometry.
Nuclear Magnetic Resonance

Spectrum 2.1.4.3. Compound $^1$H NMR (300 MHz, DMSO-$d_6$).
Spectrum 2.1.4.4. Compound 7 $^{13}$C NMR (500 MHz, DMSO-$d_6$).
Spectrum 2.1.4.5. Compound 8 $^1$H NMR (300 MHz, DMSO-d$_6$).

![NMR Spectrum of Compound 8](image)
Spectrum 2.1.4.6. Compound 8 $^{13}$C NMR (500 MHz, DMSO-$d_6$).
2.1.5 Testing of the acetylated benzothiadiazines

By measuring the change of distance between the two dyes labeled at the 5’terminals using FRET assay, we were able to evaluate the bioactivity of the potential inhibitors towards binding with IIa subdomain of HCV IRES. While the FRET assay for these two compounds indicates that the compounds may have no binding affinity with the IIaRNA under pH 7, the compounds display capability of conformationallymanipulating the IIa subdomain at pH 5.5.

Figure 2.1.5.1. Normalized FRET signals of Cy3/Cy5-labelled HCV IIa RNA titrated with compound 7 (red dots) and 8 (black dots). The EC$_{50}$ for compound 8 is 5µM.
Compound 5 has an EC\textsubscript{50} of 11 ± 7 µM, while after acetylation at the 3-amino group, the binding affinity dropped significantly to nearly no effect. However, the compound 8 has a higher binding affinity towards the binding pocket than that of the compound 7. The EC\textsubscript{50} for compound 8 is 5µM. These indicate that the hydrogen at the 3-amino group and the 2-N of the thiadiazine ring are crucial for a correct binding. This could be regarded as a proof for the importance of the ligands’ tautomerization when bonding with IIa subdomain; compound 7 has 2 tautomers (Figure 2.1.5.2A), whereas compound 8 has 3 (Figure 2.1.5.2B). Among these structures, compound 8 is the only compound that has a tautomer with hydrogen residing both on the 3-amino group and the 2-N of the thiadiazinering. The improved binding affinity for compound 8 at pH5.5 compared to that of pH 7 further supports the hypothesis. The protonated compound is equipped with the required hydrogen to target the IIa subdomain, and create hydrogen bonds when bonding with the G110 (Figure 1.6.1C) (Figure 2.1.5.3).
Figure 2.1.5.2 (A). Compound 7 tautomers. (B) Compound 8 tautomers contributing hydrogen bonding at G110 of HCV IRES IIa subdomain.
Figure 2.1.5.3. Schematic of the interactions between compound 8 and the IIa subdomain in the ligand-binding site.

2.2 Synthesis for di-substituted benzothiadiazine

With the non-planar sulfonyl group in position, we were curious to see how does the substitution at 6-postion of the benzothiadiazine scaffold could affect the interaction between the ligand and the binding pocket at the IIa subdomain.
2.2.1 1st synthetic route using 2,4-di-nitrobenzenesulfonylchloride as a starting material

The first proposed synthetic route toward the formation of our desired compound was illustrated in Scheme 2.2.1.1. Our initial plan depends critically on the reduction reaction for the nitro groups.

Scheme 2.2.1.1. The first proposed route for the synthesis of di-substituted benzothiadiazine9
2.2.1.1 Amidation reaction for 2,4-di-nitrobenzenesulfonylchloride

The starting material was treated with an excess amount of ammonium hydroxide (28%-30%) over ice bath. However, the purification process for the reaction was not successful, and the break down product aniline is mixed with our desired product.

![Chemical structure](attachment:image.png)

Scheme 2.2.1.1.1. Amidation reaction.
b: ammonium hydroxide, acetone, 0 °C, 0.5h

The purification process is complex and the yield from this reaction is low (below 10%). Since the amidation reaction is the first step of this multi-step synthetic route, the low yield made us to abandon the route, and worked on figuring out an alternative way for the synthesis.
2.2.2 2nd synthetic route using 4-chloro-2-nitrobenzenesulfonylchloride as a starting material

The 2nd synthetic route we came up with is shown in the Scheme 2.2.2.1.

Scheme 2.2.2.1. An alternative synthetic route for synthesizing of compound 9

2.2.2.1 Amidation for 4-chloro-2-nitrobenzenesulfonylchloride

An excess amount of ammonium hydroxide (28%-30%) was added to an acetone solution of the starting material over ice bath. The product from the reaction could be easily filtered out after being neutralized by the hydrochloric acid to a pH of 7. The desired product is a white solid, and the yield for this
reaction is about 85% (Scheme 2.2.2.1.1).

![Reaction diagram]

Scheme 2.2.2.1.1. Amidation reaction for 4-chloro-2-nitrobenzenesulfonyl chloride
b: ammonium hydroxide, acetone, 0 °C, 0.5h

2.2.2.2 Reduction for 4-chloro-2-nitrobenzenesulfonamide

To reduce the nitro group of the 4-chloro-2-nitrobenzenesulfonamide, we tried to use hydrazine as a reagent first, however, the conversion from the starting material was not obvious, and due to the safety issue when dealing with hydrazine, we went to seek help from Pd/C catalyzed hydrogenation reaction.

4-chloro-2-nitrobenzenesulfonamide was treated with hydrogen in methanol with a catalytic amount of Pd/C. The reaction affords 2-amino-4-chlorobenzensulfonamide with a yield of about 10%. 
Scheme 2.2.2.2.1. Pd/C catalyzed hydrogenation reaction.
c: Pd/C (10 mol%), Methanol, 12 hours.

Since the procedure was originally done under pressure according to Vanier GS, yet we performed the reaction under normal pressure, the conversion rate was not applicable for the next several steps of this route.

2.2.3 The third synthetic route

To circumvent the reduction reaction for the nitro group on the benzene ring, we decided to take advantage of the copper catalyzed Ullmann reaction for a direct coupling of the amino group.
2.2.3.1 Copper catalyzed Ullmann reaction

We started by using copper powder as a catalyst. However, the conversion rate from the starting material is low and erratic, and we could not purify the compound from the crude product. The starting material 4-bromo-2-chlorobenzensulfonamide tends to form a self-coupled product under 80 °C, thus, we adjusted the temperature to 60 °C for avoiding the side product. Knowing that the active species in the catalytic cycle of the reaction is Cu (I),\(^{33}\) we decided to use copper (I) oxide as a catalyst. By optimizing the reaction time and
temperature, we were able to perform the reaction with a yield of about 50% (Table 2.2.3.1.1).

Table 2.2.3.1.1. Various conditions for Ullmann reaction of 4-bromo-2-chlorobenzenesulfonamide.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Atmosphere</th>
<th>Catalyst (mol %)</th>
<th>Temperature (°C)</th>
<th>Time (h)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>air</td>
<td>Cu (2)</td>
<td>80</td>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>air</td>
<td>Cu (3)</td>
<td>60</td>
<td>6</td>
<td>n.a.</td>
</tr>
<tr>
<td>3</td>
<td>air</td>
<td>Cu (3)</td>
<td>60</td>
<td>16</td>
<td>n.a.</td>
</tr>
<tr>
<td>4</td>
<td>air</td>
<td>Cu (3)</td>
<td>60</td>
<td>44</td>
<td>n.a.</td>
</tr>
<tr>
<td>5</td>
<td>argon</td>
<td>Cu₂O (2)</td>
<td>60</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>6</td>
<td>argon</td>
<td>Cu₂O (5)</td>
<td>60</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>7</td>
<td>argon</td>
<td>Cu₂O (5)</td>
<td>60</td>
<td>48</td>
<td>50</td>
</tr>
</tbody>
</table>

The original research done by JiaoJiao et al. indicated that amines favored bromo-group when attacking a 4-bromo-2-chloro benzene,³³ yet we obtained a chloro-group favored result. This could be contributed from the coordination
between the 3-(dimethylamino)-1-propylamine and the sulfonamide group of the compound.

2.2.3.2 Cyclization for 4-bromo-2-((3-(dimethylamino)propyl)amino)benzenesulfonamide

With the product 10 acquired from the previous step, we were able to explore the procedures for cyclizing the compound.

We performed the reaction by heating the compound 10 and guanidine carbonate together at 200 °C. However, the compound was not capable of sustaining a high temperature and we could not get the desired product from this reaction (Scheme 2.2.3.2.1).
Scheme 2.2.3.2.1. Melting reaction with guanidine carbonate at 200°C.

We also tried using cyanogen bromide as a cyclizing reagent. We were able to see some conversion for the desired product according to the mass spectrometry, yet most of the starting material 10 was still left unreacted.

Scheme 2.2.3.2.2. Cyclization reaction using BrCN.

\[
\begin{align*}
\text{BrCN} & \quad \text{d: CH}_3\text{OH (anhydrous), argon, 0°C to room temperature, 13 hours.}
\end{align*}
\]

2.2.4 Materials and methods

2.2.4.1 Amidation reaction for 4-chloro-2-nitrobenzenesulfonylchloride
Ammonium hydroxide (29.6 mmol, 2 mL) was added into a round bottle flask with magnetic stirring in an ice bath. An acetone solution (15 mL) of 4-chloro-2-nitrobenzenesulfonylchloride (3.2 mmol, 822.9 mg) was added into the round bottle flask dropwise. 30 minutes later, the solvent was removed under reduced pressure, and 37 % hydrochloric acid was added to the crude. The pure product 4-chloro-2-nitrobenzenesulfonamide was filtered out as a white solid.

2.2.4.2 Copper (I) oxide catalyzed Ullmann reaction

3-(dimethylamino)-1-propylamine (4 mmol, 0.5 mL), 37% hydrochloric acid (1.2 mmol, 0.1 mL), Cu$_2$O powder (0.25 mmol, 37 mg), 4-bromo-2-chlorobenzenesulfonamide (0.7 mmol, 180 mg) was added to 6 mL of degassed methanol. The mixture was magnetically stirred in an argon system under 60 °C for 48 hours. For the purification process, the solvent was removed in vacuo, water and ethyl acetate was added for extraction. 37% HCl was added to the organic phase for a pH of 5, and the pure product was extracted from the
organic solution by adding water. The aqueous solvent was removed under
reduced pressure to afford the pure compound 10.

10: $^{1}$H NMR (300 MHz, DMSO-d$_6$)$\delta$(ppm): 8.475 (s, 1H), 7.604 (s, 1H), 7.495 (s,
1H), 6.612 (s, 1H), 3.055 (t, 2H, J = 6.92 Hz), 2.188 (t, 2H, J = 7.19 Hz), 2.086 (m,
6H), 1.659 (m, 2H); $^{13}$C NMR$\delta$(ppm): 132.511, 131.627, 129.752, 121.252,
116.196, 56.963, 48.908, 45.641, 26.915; HRMS mass calculated C$_{11}$H$_{18}$BrN$_3$O$_2$S
[M+H]$^+ = 336.0376$, found 336.0375, delta -0.3 ppm.
2.2.5 Spectral data

Mass spectrometry

Spectrum 2.5.1 Compound 10 high resolution mass spectrometry.
Nuclear Magnetic Resonance

Spectrum 2.5.2 Compound $^{1}H$ NMR (300 MHz, DMSO-$d_6$).
Spectrum 2.5.3 Compound 10$^{13}$C NMR (500 MHz, DMSO-d$_6$).
References


(10) Dore, G. J.; Podsadecki, T. **2015**, *1*.


