Stony Brook University

Design and Synthesis of α-Truxillic Acid-Based Fatty Acid Binding Proteins Inhibitors

A Thesis Presented

by

Qianwen Gan

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Qianwen Gan

We, the thesis committee for the above candidate for the

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Abstract of the Thesis

Design and Synthesis of α-Truxillic Acid-Based Fatty Acid Binding Proteins Inhibitors

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Pain management has become a major issue in health care. It has been reported that about 100 million Americans are affected by chronic pain, and it costs us about $600 million per year. Current drugs used in pain management such as opioids, and marijuana have some unwanted side effects. Thus, there is a need to explore and develop novel, safer and more effective drugs for pain relief. Anandamide (AEA) is an endocannabinoid, and it can activate cannabinoid(CB) receptors on the cell surface, resulting in pain relief. However, AEA can also diffuse into cells, transported by fatty acid binding proteins (FABPs) to fatty acid amide hydrolase (FAAH) where AEA is hydrolyzed. FABP inhibitors can block the pathway for AEA to be inactivated, resulting in enhancement of the pathway to relief pain. Our interdisciplinary research team has determined the co-crystal structures of FABP5 and FABP7 with our previous lead compound SB-FI-26. Based on the co-crystal structure of the FABP5-SB-FI-26, new SB-FI-26 analogues have been designed and synthesized to optimize potency. The design and synthesis of α-truxillic acid derivatives, as well as their biological evaluations will be presented.

In addition, the synthesis of novel matrix metalloproteinase (MMP) 9 inhibitors will also be presented.

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Chapter 2

Matrix metalloproteinase-9 inhibitors

List of Abbreviations

2-AG 2-Arachidonoylglycerol

AcCN Acetonitrile

AEA Anandamide

CB receptor Cannabinoid receptor

^{13}C NMR Carbon-13 nuclear magnetic resonance

DCM Dichloromethane

DIPEA Diisopropylethylamine

DMAP Dimethylaminopyidine

DMF Dimethylformamide

DMSO Dimethylsulfoxide

EDC·HCl 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride

FAAH Fatty acid amide hydrolase

FABP Fatty acid binding protein

FDA Food and drug administration

FIA Flow injection analysis

HCl Hydrochloric acid

^{1}H NMR Proton nuclear magnetic resonance

HPLC High pressure liquid chromatography

HRMA High resolution mass spectrometry

IP Intraperitoneal

mp Melting point

MeOH Methanol

MMP Matrix metalloproteinase

NBD-stearate 12-N-methyl-(7-nitrobenz-2-oxa-1,3-diazo)aminostearic acid

SAR Structure-activity relationship

TEA Triethylamine

TFA Trifluoroacetic acid

THF Tetrahydrofuran

TLC Thin layer chromatography

UV Ultraviolet

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Chapter 1

Antinociceptive Agents Targeting Fatty Acid Binding Proteins(FABP)

1.1 Introduction

1.1.1 Pain management and fatty acid binding proteins (FABPs) inhibitors

Pain is associated with a wide range of actual or potential tissue damage, or sometimes described in terms of such damage. There are two kinds of pains, acute pain and chronic pain. Acute pain is protective, when acute pain occur people can notice the damage from environment and then take care of themselves. However, chronic pain outlives its usefulness as an acute warning system, it lasts for weeks, months, even years.[1, 2] According to a research report more than 1.5 billion people are affected by chronic pain of varying degrees, and around 3-4.5% of the global population suffer from neuropathic pain which is one type of chronic pain.[3] In addition, about 100 million American adults are affected by chronic pain, and it costs about $600 million per year for the treatment and loss of productivity. These results indicate that pain management has become a major issue in healthcare.

Each kind of current drugs used in pain management have some undesired side effects. Opioids are regard as strong drugs for pain management, they act by binding to opioid receptors, which are located in both the central nervous system and the peripheral nervous system. Although the use of opioids can provide patients with a narcotic effect which induce pain relief, the side effect can not be ignored. Opioids can cause drowsiness, dizziness, respiratory depression, and there is a risk lead to addiction or dependence.[4] Another pain medication is cannabinoid, such as delta-9-tetrahydrocannabinol(THC) which is the active component in marijuana. THC can

activate the G-protein-coupled cannabinoid receptor (CB1), resulting in anti-nociceptive and anti-inflammatory effects.[5]However, the THC has some side effects including somatic effects and psychological effects, such as increasing heart rate, dry mouth, auditory as well as visual hallucination.[6] Unlike THC is extracted from plants, Endocannabinoids are biosynthesized in mammalian body. This endogenous agonist of CB receptors has similar pharmacological effects to that of THC. However, its activity is limited by cellular uptake and then inactivated by fatty acid amide hydrolase.[7] Hence regulating pain by inhibiting the degradation pathway of endocannabinoids can be a probable method to regulate pain without psychoactive effects.

The endocannabinoid anandamide is biosynthesized in a small amount and is degraded by fatty acid amide hydrolase (FAAH) which is located on endoplasmic reticulum. Since anandamide is an uncharged hydrophobic molecule, it can diffuse into the cell member, but an intracellular carrier is needed to transport it to the endoplasmic reticulum through the aqueous environment of the cytoplasm.[8]Fatty acid binding proteins (FABPs) are a family of transport proteins to shuttle fatty acids and some other lipophilic substances throughout the cells.[9, 10] FABPs are tissue specific, FABP3 are expressed in the heart, FABP5(epidermal FABP) are expressed in the epidermal, FABP7(brain FABP) are expressed in brain. It has been identified that fatty acid binding protein, especially FABP5 and FABP7 act as intracellular transport for the anandamide, they transport AEA for degradation in FAAH (**Figure 1**). Based on these, FABP5 and FABP7 could be pharmacological targets. The inhibitors of FABPs can decrease the transportation of the AEA to FAAH to halt the hydrolysis of AEA, results in raising extracellular anandamide and anti-inflammatory and anti-nociceptive effects. [8, 11]

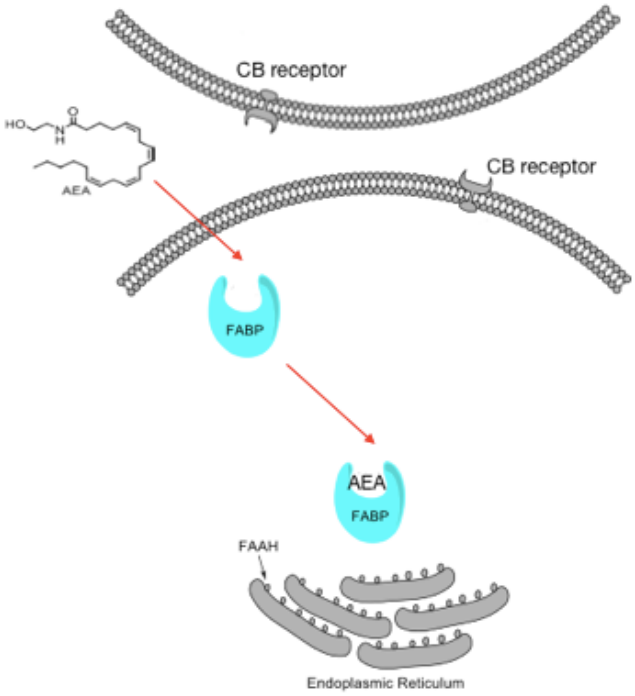


Figure 1 Anandamide (AEA) can enter the cell by diffusion, transported by fatty acid binding proteins (FABPs), followed by inactivated by fatty acid amid hydrolase (FAAH), or activate cannabinoid receptor results in anti-nociceptive and anti-inflammatory effects. Inhibition of FABPs can arrest the degradation of AEA, resulting in pain relief effect.

1.1.2 Discovery of α-traxillic acid derivatives

1.1.2.1 In-silico screening

In order to identify novel FABP inhibitors, Footprint similarity(FPS) score, a new scoring function based on per-reside van der waals, electrostatic, and hydrogen bond energies was used to identify FABP inhibitors with profound binding affinity with FABPs. Unlike traditional DOCK which uses a two-term score to rank binding affinity of ligands with a target, FPS takes energy score and decomposed it into per-residue contributions to get a distinct signature between the target

and ligand.[8, 12] If the FPS score of the inhibitor candidates are similar to the nature ligand of FABPs, it indicates that these compound show a high binding affinity to FABPs. [12]

Since CB-1 receptor is mainly expressed in the brain, FABP5 and FABP7 are considered target FABPs. FABP5 is epidermal FABP, it is present throughout the body including dendritic cell, tongue, adipose tissue, brain neurons, kidney, lung, liver and it present abundantly in the epidermal cells of the skin. FABP7, brain FABP, is expressed during midterm embryonic development. Both FABP5 and FABP7 show high binding affinity with fatty acid, and the co-crystal of FABP7 and olieic acid have been discovered. Hence, FABP7 is the one used for virtual screening. [8]

Oleic acid is a nature ligand of FABP7, was used as the reference molecule in the high-throughput virtual screening. After screening one million commercially available compounds from ChemDiv, and comparing their footprint similarity score to that of oleic acid, 48 compounds were found have similar overlap in FPS score of oleic acid. This overlap means screened compound is binding to FABP7 in a similar way to oleic acid (**Figure 2**), indicating the tested compound interacted with the same amino acid as oleic acid in the binding site of FABP7. According to the results of virtual screening, 48 compounds were purchased and assayed in-vitro study subsequently.[8]

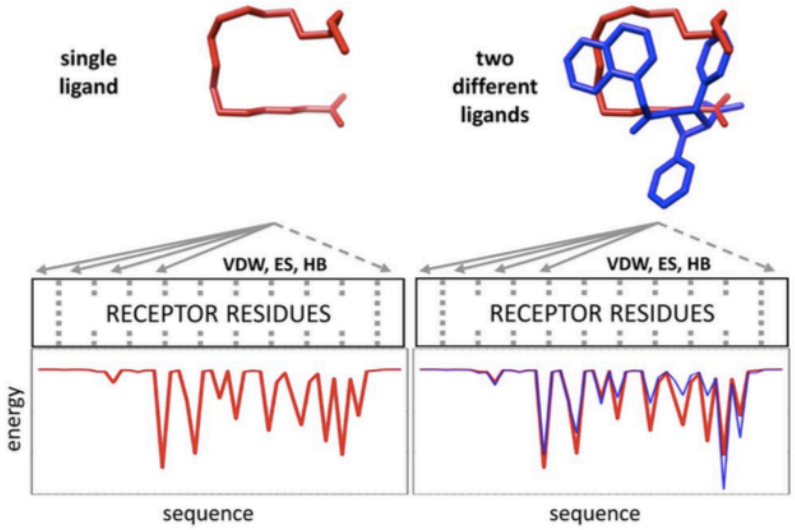


Figure 2 Footprint signature of tested compound is compared to the footprint signature of oleic acid. (Copied from Ref. [8])

1.1.2.2 Fluorescence displacement assay

According to the result of the in-silico screening to FABP7, 48 lead compounds were purchased and assayed in-vitro study subsequently against FABP5. Compared to FABP7, FABP5 was chosen because of its ease of expression. The fluorescence displacement assay of the 48 lead compounds (10 μM) displaced NBD-stearate (1 μM) from FABPs is shown is **Figure 3**. The Buffer and NBD-stearate don't show high fluorescence intensity, but NBD-stearate with purified FABP5 shows a high fluorescence signal. The positive control is the forth sample arachidonic acid (1 μM) which has a high binding affinity with FABP, it decreased the signal when binding with FABP5.[8] For the fluorescence displacement, the lower intensity means the higher binding affinity. The intensity result indicated that about one-third of the test compounds lead to a decrease in fluorescence and four of the most potent compound that showed at least 50% inhibition were chosen for further

investigation (**Figure 4**). These four compounds all have a carboxylate moiety, which is similar to oleic acid, hence the carboxylate group is determined to be important for binding with FABP5.

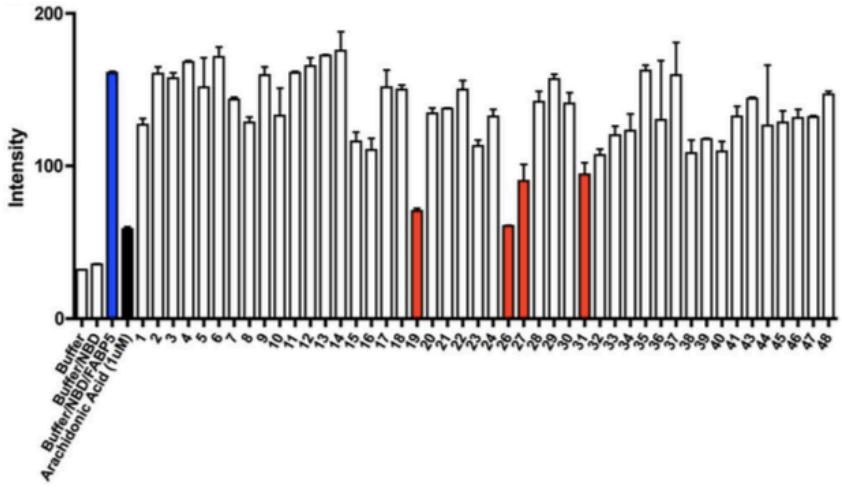


Figure 4 Fluorescence displacement assay of 48 compounds with NBD-stearate. NBD-stearate with purified FABP5 (blue), the positive control (black) arachidonic acid, the four leads comounds (red) showed at least 50% inhibition. (Copied from Ref. [8])

These four compounds: SB-FI-19, SB-FI-26, SB-FI-27, SB-FI-31 (**Figure 5**) were then send to rerun in the NDB-fluorescent displacement assay at 10 μM. However, one of the compounds SB-FI-26, α-truxillic acid 1-naphthyl mono-ester, was incorrectly provided by the vendor as the gamma truxillic acid derivative (SB-FI-49). Hence, SB-FI-26 and SB-FI-49 were synthesized and retested in-vitro. After triplicate analyzing, SB-FI-49 and SB-FI-26 exhibited good binding affinity with FABP5. SB-FI-26 showed a Ki value of 0.93±0.08 μM, which is higher than the Ki value of SB-FI-49 which is 0.75±0.07 μM.[8, 11] However, SB-FI-49 was less soluble (200 μM in DMSO, 25 ^{o}C) than SB-FI-26 (1 mM in DMSO, 25 ^{o}C). Hence, SB-FI-26 was chosen for further development as a FABP inhibitor.

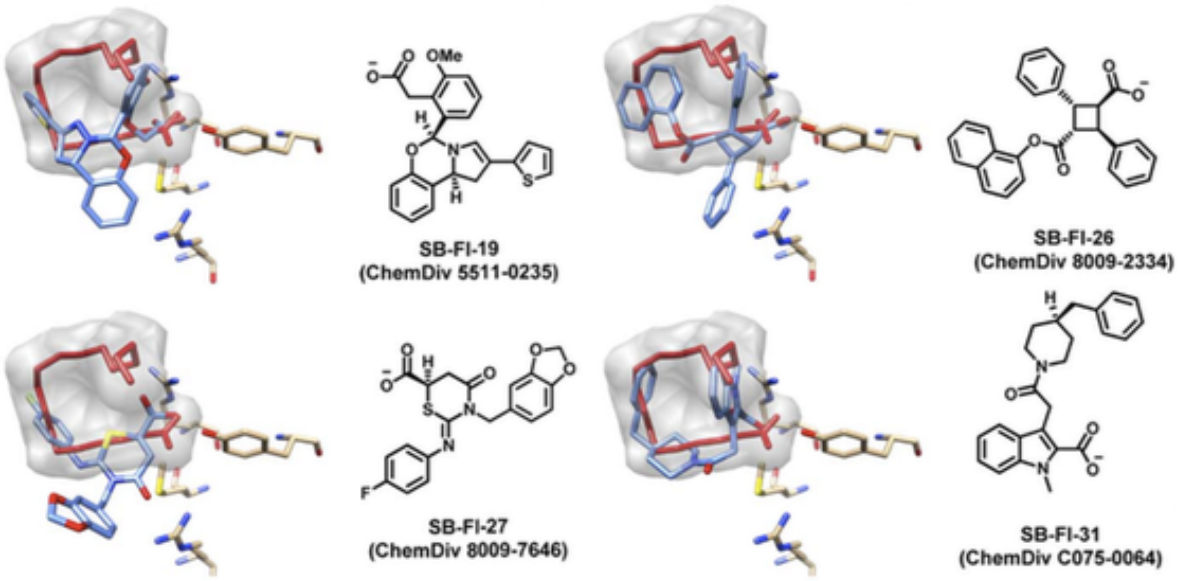


Figure 5 Four lead compounds from the FABP7 virtual screen and fluorescence displacement assay. Left, the predicted binding pose of each compounds (blue) is related to the oleic acid (red). (Copied from Ref. [8])

1.1.2.3 Antinociceptive and anti-inflammatory effects of SB-FI-26

Since inhibition of FABPs can arrest the transportation of AEA to FAAH for inactivation, FABP inhibitors might lead to antinociceptive and anti-inflammatory effects. Two nociceptive models, the formalin test as well as carrageenan-induced thermal hyperalgesia, are used to exam the effects of SB-FI-26. The formalin test including two phases, the first one is from zero to five minutes, the second phase is from fifteen to forty-five minutes, reflecting nociceptor activation and an inflammatory pain response respectively. During the first phase, SB-FI-26 reduced nocifensive behavior significantly (**Figure 6A**). [6-8]

Carrageenan-induced thermal hyperalgesia test was used to explore whether SB-FI-26 can relief inflammatory pain. As show in the **Figure 6B**, SB-FI-26 reduced thermal hyperalgesia and paw edema significantly. To figure out whether this action is caused by a cannabinoid receptor-mediated mechanism, rimonabant and SR144528 which are the antagonists of cannabinoid receptor 1 and 2, were pretreated on mice. According to the test results, the reduction of thermal hyperalgesia and paw edema caused by SB-FI-26 were completely reversed by the cannabinoid receptor 1 and 2 antagonists **(Figure 6B and C**). This result indicated that the inhibition of FABPs produces antonociceptive and anti-inflammatory effects, and those effects are mediated by cannabinoid receptors.[6-8]

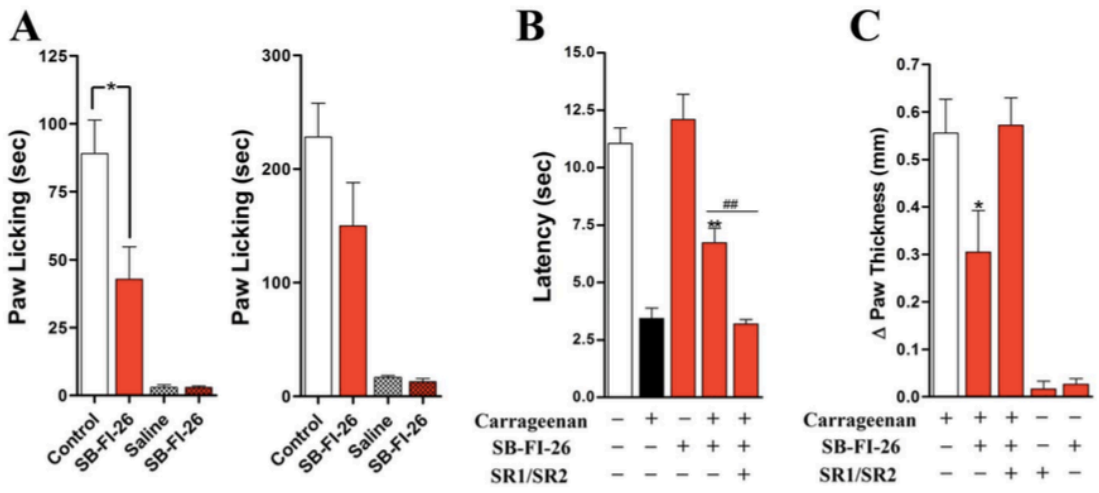


Figure 6 Antinociceptive and anti-inflammatory effects of SB-FI-26 (A) Effect of SB-FI-26 (20 mg/kg) on the first (left) and second phase (right) of formalin test. Time spend on licking and biting is record for 60 min. (B) Effects of SB-FI-26 (20 mg/kg) on carrageenan-induced thermal hyperalgesia, administration of SR1/SR2 reversed the effects of SB-FI-26. (C) Effects of SB-FI-26 (20 mg/kg) on carrageenan-induced paw edema. (Copied from Ref. [8])

1.1.2.3 *In-vivo* study of α-truxillic acid derivatives.

Three analogs based on SB-FI-26 were synthesized to identify the FABP subtypes mediating antinociceptive effects. They are SB-FI-50, SB-FI-60, SB-FI-62. SB-FI-50 was a 2-naphthol monoester, it is used to explore if different functional group on the ester can lead to a different binding affinity.[13] Since the ester was consider to be too astable, to find more stable functional groups 1-naphthylamine monoamide (SB-FI-60) and diamide (SB-FI-62) were synthesized.[13] In the computational analysis, these analogues of SB-FI-26 have higher binding affinity against FABP7 than FABP3 or FABP5, which in agreement with the experimental results. (**table 1**) Since SB-FI-62 doesn't have the carboxylic acid which was thought to be essential for interaction affinity, it has low binding affinity for FABP3, FABP5, FABP7. Compared with SB-FI-26, SB-FI-50 and SB-FI-60 have lower affinities for FABP5, similar affinities for FABP7 and low affinities for FABP3.[13]

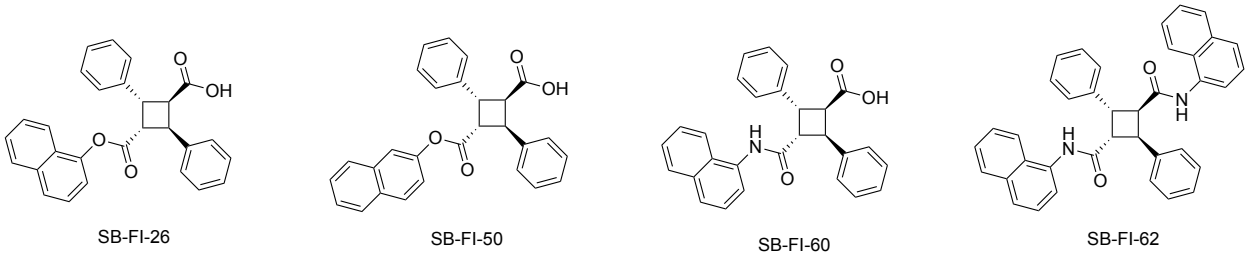


Figure 7 Strudctures of SB-FI-26, SB-FI-50, SB-FI-60, SB-FI-62.

Table 1 Binding affinities of SB-FI-26, SB-FI-50, SB-FI-60, SB-FI-62 to FABPs.

Compound	FABP3	FABP5	FABP7
	K_i (μM)	K_i (μM)	K_i (μM)
SBFI26	3.9±0.7	0.9±0.1	0.4±0.0
SBFI50	3.5±0.3	1.3±0.2	0.6±0.1
SBFI60	>10	1.6±0.0	0.3 ±0.0
SBFI62	2.6±1.1	3.3±0.7	6.1±0.5

The K_i values represent averages ± S.E. of three independent experiments.
doi:10.1371/journal.pone.0094200.t002
(Copied from Ref. [13])

Several pain models were used to test the pain reduce effects of SB-FI-26, SB-FI-50, SB-FI-60, as well as SB-FI-62. In the carrageenan model, these four compounds were dissolved in DMSO and administered on mice though intraperitoneal (i.p.). After 60 min, 1% λ-carrageenan was injected into the plantar surface of paws. Then thermal hyperalgesia as well as paw edema were measured. Hyperalgesia and paw edema were reduced by SB-FI-26 and SB-FI-50, but the other two analogs were ineffective. In the formalin model, SB-FI-26, SB-FI-50 as well as Sb-FI-60 reduced pain in the first phase which involves nociception pain, but only SB-FI-26 reduced pain in the second phase which involves inflammation. And SB-FI-62 was ineffective in this formalin test. For visceral pain, acetic acid writhing model was used to exam the antinociceptive effects of these inhibitors. α-truxillic acid derivatives were injected subcutaneously followed by acetic acid. It demonstrated that only SB-FI-26 significantly reduced the writhing caused by acetic acid. Above all, SB-FI-26 was the best compound among these tested inhibitors, it showed effects on reducing inflammatory, visceral, as well as neuropathic pain, while other compounds were locking efficacy.[13]

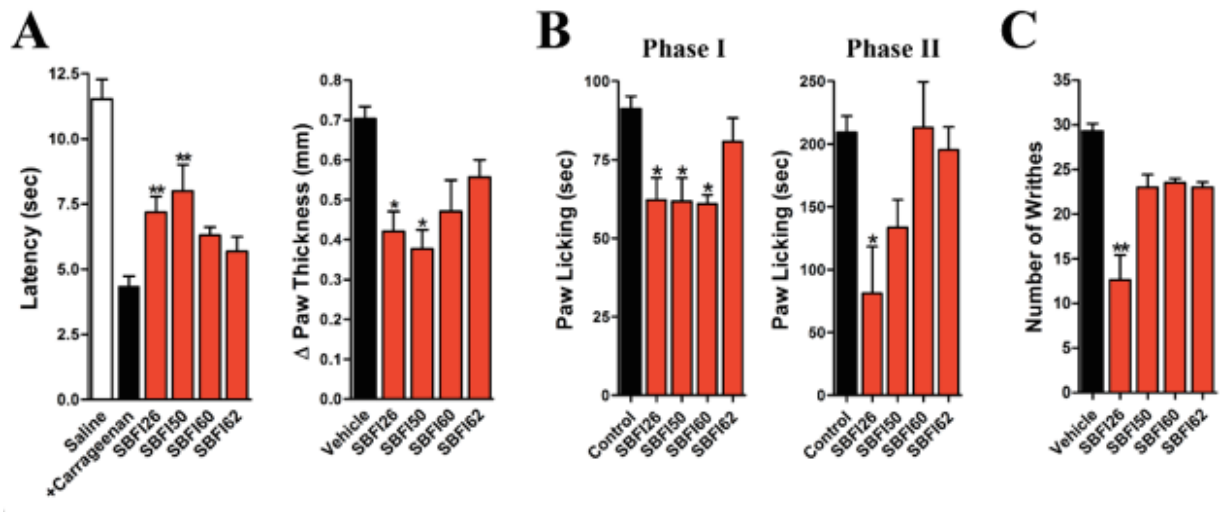


Figure 8 a) Effects of SB-FI-26, SB-FI-50, SB-FI-60 and Sb-FI-62 on carrageenan induced hyperalgesia (left) and paw edema (right) in mice. b) Effects of FABP inhibitors on formalin inflammatory pain model in mice. Left, first phase, right, second phase. C) Effects of FABP inhibitors on acetic acid induced visceral pain model in mice. (Adapted from Ref.[13])

1.2 Design and Synthesis of α-truxillic acid derivatives

1.2.1 Design of α-truxillic acid derivatives

Mono ester synthesis and aromatic ring modification of SB-FI-26 are focused on to synthesize new compound. For instance, the carboxylic acid of SB-FI-26 was substituted by 2H-Pyran-4-methanol and the phenyl rings were substituted by 2-pyridy. Based on the study from Li group, the co-crystal structure of SB-FI-26 bound to FABP5 was obtained, AutoDock have been used to choose which analogues of SB-FI-26 are worth being synthesized. AutoDock can identify the binding affinity between the tested compound and FABP5 as well as calculate the position of tested compound compared with SB-FI-26 in the binding pocket. After calculation, a binding energy of the tested compound to FABP5 would be obtained, if the binding affinity is similar to or higher

that of SB-FI-26 to FABP5 (**Figure 9**), chances that the tested compound may be a good FABP inhibitor and worth being synthesized. Hundreds of compounds have been tested, some calculation results are listed in **Table 2**. Besides binding energy, cLogP was took into consideration as well. The cLogP is an established measure of the compound's hydrophilicity, it is the logarithm of compound's partition coefficient between n-octanol and water. Compounds with high cLogP value are tend to express more *in vivo* toxicity.[14] The result of docking and cLogP were used to optimize compounds for low binding energy, low toxicity, and good solubility.

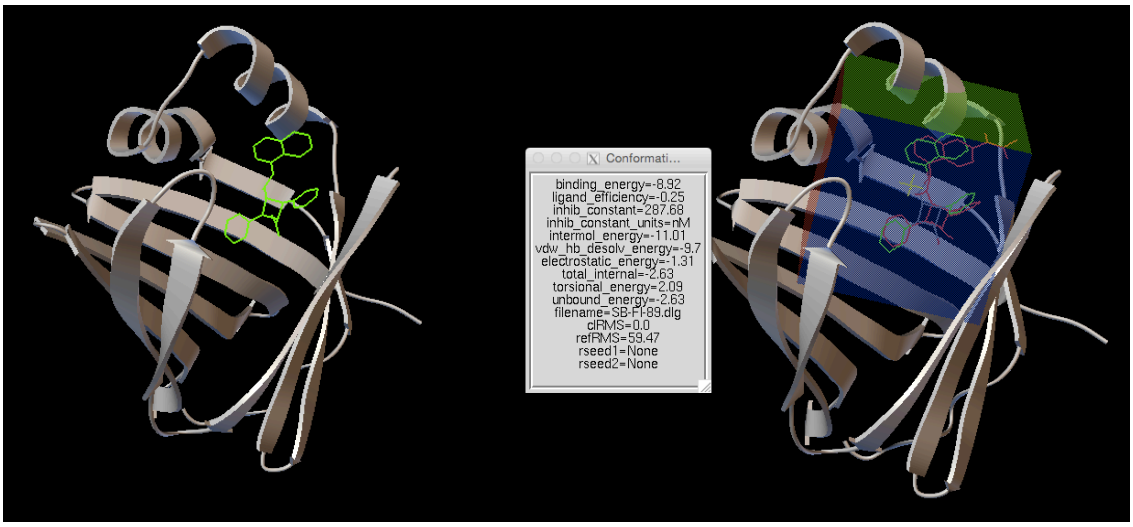
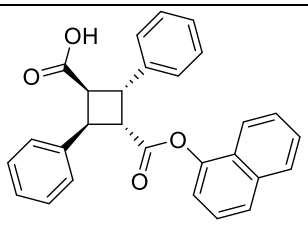
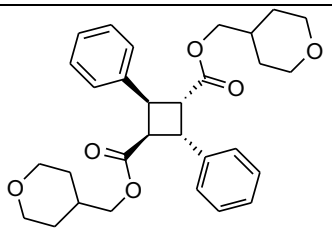
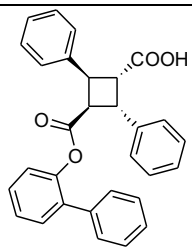
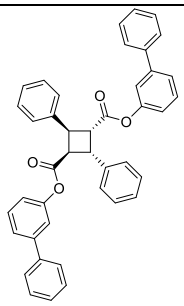
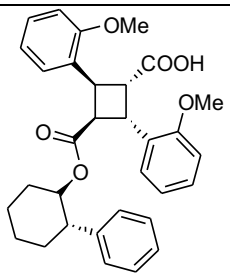
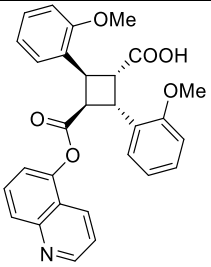
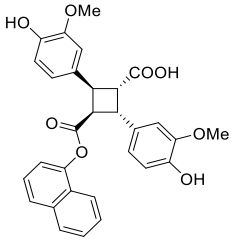
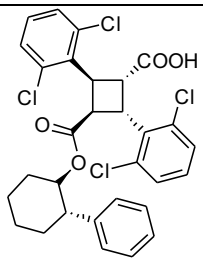
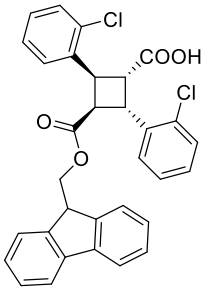
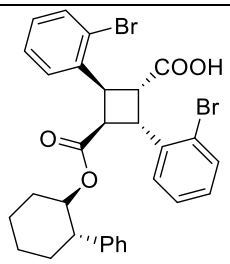
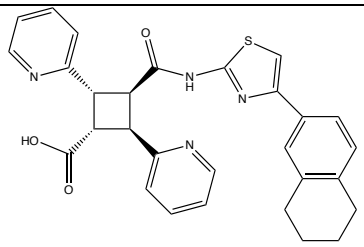


Figure 9 The co-crystal structure of SB-FI-26 bound to FABP5 in AutoDock (Left). (Co-crystal structure are provided by Huilin Li's group). A tested compound overlaps with SB-FI-26 in the binding site of FABP5 in docked structure.

Table 2. Docking score to FABP5 (AutoDock)

code	Structure	Docking Score (kcal/mol)	cLogP
SB-FI-26		-8.26	5.96
SB-FI-86		-6.85	3.968
SB-FI-91		-8.75	6.119
SB-FI-401		-8.47	10.29

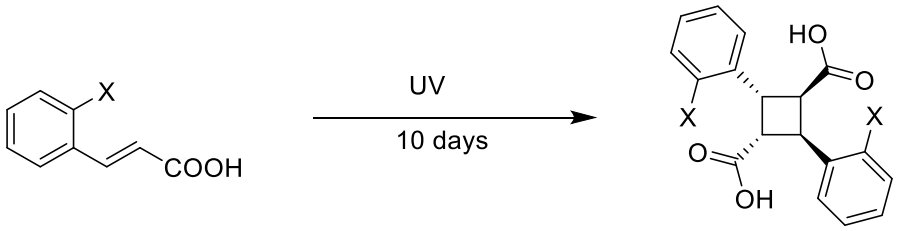
SB-FI-104		-7.99	6.203
SB-FI-105		-8.42	4.89
SB-FI-101		-7.04	4.329
SB-FI-200		-8.49	10.017
SB-FI-202		-8.76	8.523

SB-FI-210		-9.09	8.891
G0034		-8.90	5.04

1.2.2 Synthesis of α-truxillic acid derivatives

According to the result of docking and cLogP, some compounds show good binding affinity with FABP5 and acceptable cLogP. To synthesize those compounds, α-truxillic acid and a series of analogues of α-truxillic acid were synthesized. The general synthesis of analogues of α-truxillic acid started with analogues of trans-cinnamic acid which was exposed to 360 nm UV light to generate [2+2] photocycloaddition and gave α-truxillic acid. These α-truxillic acid are used for next step to obtain analogues of SB-FI-26.

Scheme 1. Synthesis of α-truxillic acid derivatives



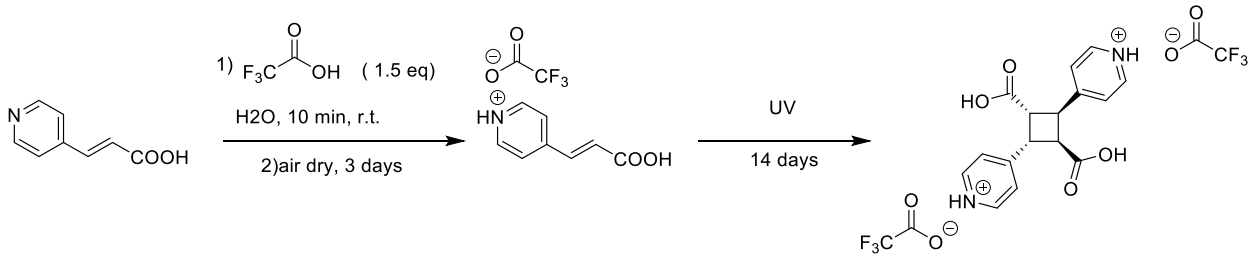
Compound Number	-X	Yield
1-1	H	55.6%
1-2	Cl	89%
1-3	OMe	62.5%
1-4	Br	67.0%

These Compounds were synthesized and used for next step to synthesize SB-FI-26 derivatives.

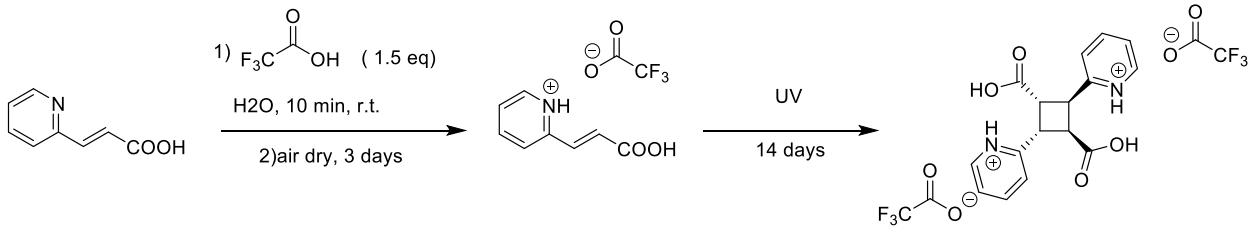
1.2.3 Synthesis of heteroaromatic α-truxillic acid

As is showed in Docking study, when the phenyl rings on α-truxillic acid were altered to heteroaromatic rings, some the derivative of SB-FI-26 showed good binding affinity and solubility. Hence, those heteroaromatic α-truxillic acid were synthesized, aimed to using as intermediate to synthesize derivative of SB-FI-26 with phenyl rings. Since pyridyl acrylic acids didn't cyclize when directly put under UV light, Trifluoroacetic acid (TFA) was used to acid activated it to improve cyclization.[15]

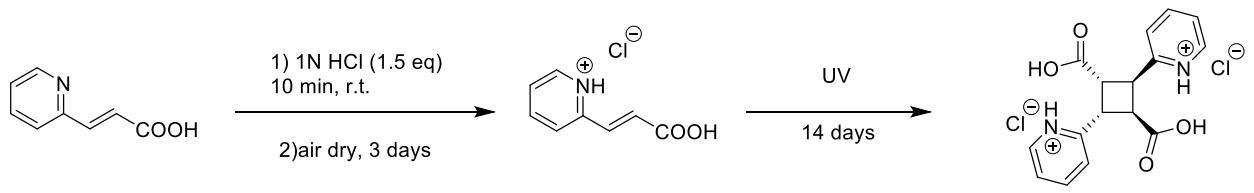
Scheme 2 Synthesis of heteroaromatic α-truxillic acid



2-1 **2-2**



2-3 **2-4**

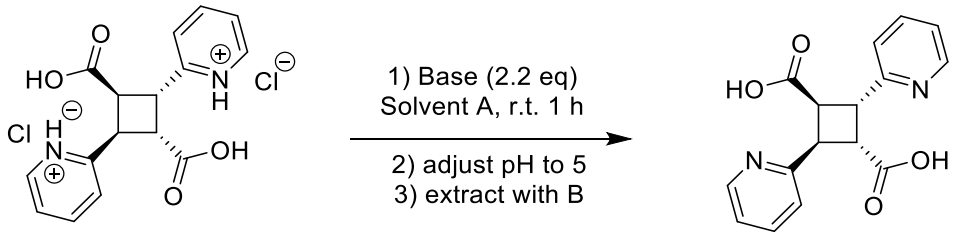


2-5

2-Pyridyl acrylic acid (**2-1**), and 4-pyridyl acrylic acid (**2-3**) were suspended in water was added TFA slowly, then the solutions were allowed to air dry to obtain the pyridinium salts **2-2**, **2-4.** The salts were put under 360 nm UV to initiate the [2+2] cycloaddition. These two products were used

to make SB-FI-26 derivative. However, when the general method was used to synthesize SB-FI-26 analogues the reaction failed many times, so trifluoroacetic acid was thought might be a reason that influence the reaction, hence hydrochloric acid was used to acid activated **2-1** and **2-5** [15]was obtained.

Scheme 3 Synthesis of α-2,4-di(pyridin-2-yl)cyclobutane-1,3-dicarboxylic acid



2-5 **3-1**

No.	Base	Solvent A	Solvent B	Extract apparatus	Yield
1	NaOH	H_2O, EA	EA	separatory funnel	25%
2	NaOH	H_2O, DCM	DCM	separatory funnel	17%
3	$NaHCO_3$	H_2O	DCM	liquid-liquid extractor	4.2%

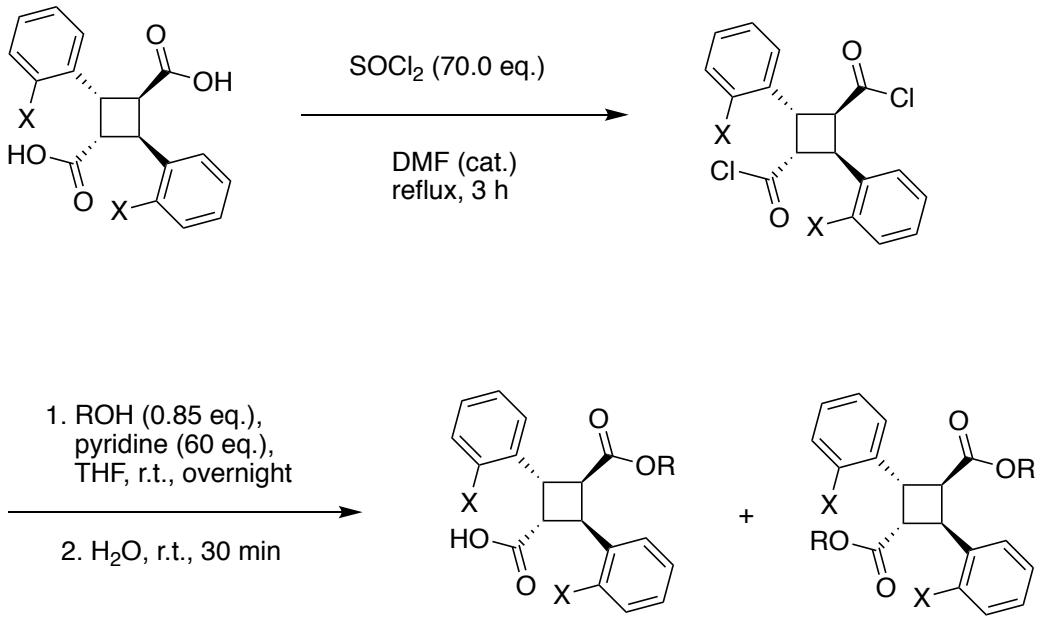
The starting material in condition **1** and **2** were 50 mg, NaOH was used to neutralize **2-5**. In condition **1**, the product was extracted by ethyl acetate (100mL*5 times), solid was obtained, but the yield was low. Condition **2** was used to find out if change extraction solvent can increase yield, DCM (100mL*5 times) was used, but the yield didn't increase. And the NMR of the product were not pure in both of them. Hence, in condition **3**, $NaHCO_3$ was used as base, and liquid-liquid extractor was used, the starting material is 150 mg, DCM (200 mL) was used to extract product

but the result indicated lower yield was obtained and NME of the product were not pure still. The reason might be the product were not fully neutralized, so clear NMR data was not obtained. And the low yield in condition **3**, indicated that it is not because there is no enough time for the solvent to extract the product from the reaction mixture, but the product was too polar to be extracted from water. Since the neutralized product can not be approach, **2-5** was used to make SB-FI-26 analogues directly.

1.3 Synthesis of SB-FI-26 derivatives

The general synthesis of SB-FI-26 derivatives started with α-truxillic acid or derivatives which was chlorinated in the present of catalytic DMF with thionyl chloride, reflux for 3 hours to obtain the diacid chloride. To identify which substitutions have a good binding affinity with FABPs, the diacid chloride was substituted with different alcohols.

Scheme 4. Synthesis of SB-FI-26 derivatives

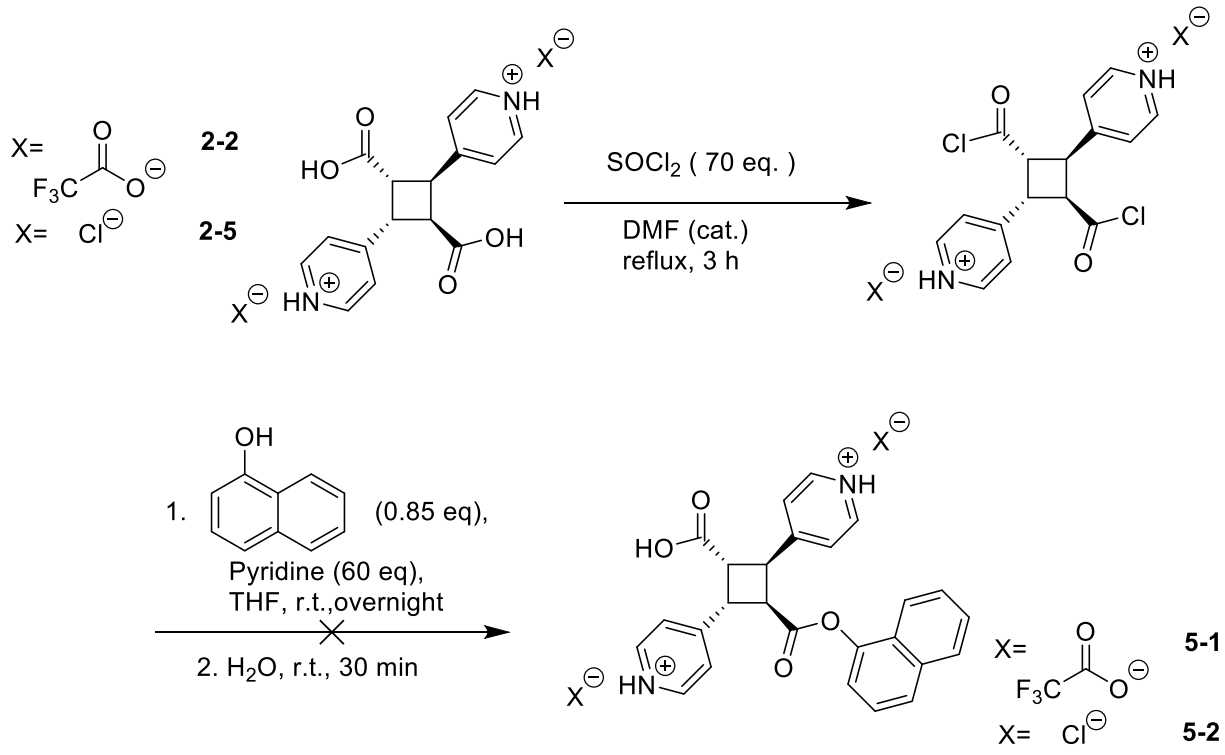


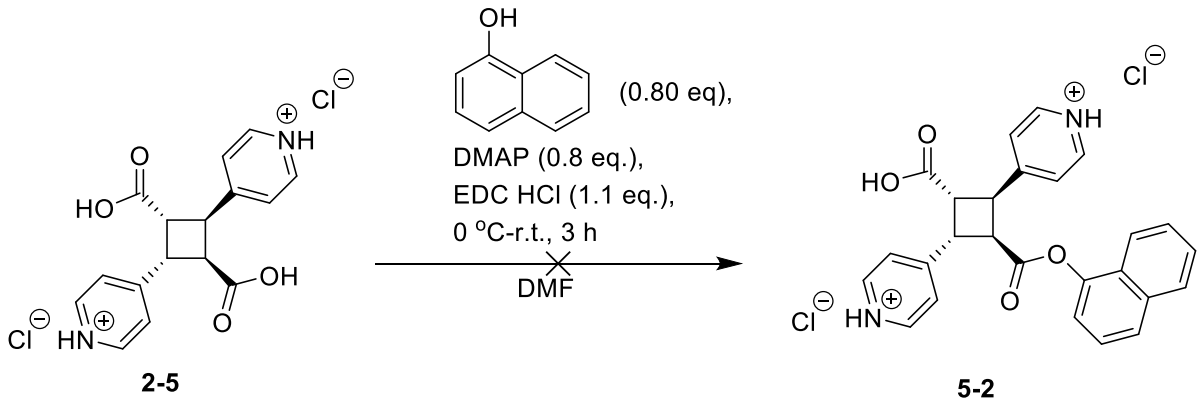
Code	X	Alcohol	yield	Ki		
				FABP3	FABP5	FABP7
SB-FI-26 **4-1**	H	naphthol	19.1 %	2.70 ± 0.42 μM	0.81 ± 0.09 μM	0.45 ± 0.07 μM
SB-FI-91 **4-2**	H	1,1'-biphenyl-2-ol	Synthesized by Yan	1.23 ± 0.71 μM	0.77 ± 0.08 μM	0.47 ± 0.01 μM
SB-FI-401 **4-3**	H	1,1'-biphenyl-3-ol (diester)	15 %	-	-	-
SB-FI-87 **4-4**	H	tetrahydropyran-4-methanol	10 %			
SB-FI-86 **4-5**	H	tetrahydropyran-4-methanol (diester)	Synthesized by Simon	>10 μM	>10 μM	>10 μM
SB-FI-105 **4-6**	OMe	5-quinolinol	29.7 %	>10 μM	3.93μM ± 0.51	>10 μM

SB-FI-104 **4-7**	OMe	(1*S*,2*S*)-2-phenylcyclohexan-1-ol	27 %	0.40 ± 0.08 μM	0.64 ± 0.07 μM	0.40 ± 0.03 μM
SB-FI-81 **4-8**	H	(1*S*,2*S*)-2-phenylcyclohexan-1-ol	Synthesized by Kongzhen	1.08 ± 0.37 μM	0.21 ± 0.02 μM	0.40 ± 0.03 μM

To synthesize heteroaromatic SB-FI-26 derivative, **2-2** and **2-5** was used as starting material, but when the same method used on α-truxillic acid with phenyl rings was applied, the reaction didn't work. Then **2-5** was used as starting material to synthesize the derivative by EDC·HCl promoted coupling, but this reaction didn't work either.

Scheme 5 synthesis of heteroaromatic SB-FI-26 derivative





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1.4 Summary

Four α-truxillic acid derivatives **1-1**, **1-2**, **1-3**, **1-4** have been synthesized and used to make SB-FI-26 derivatives.

The new lead compound, SB-FI-81, was synthesized by Kongzhen Hu. It exhibited highest binding affinity to FABPs. It shares the same core with SB-FI-26, but different alcohol were used to synthesize it. This alcohol may contribute to the highe binding affinity. Hence, SB-FI-104 was synthesized which use the same alcohol as SB-FI-81. According to the *in vivo* result, SB-FI-104 exhibited better binding affinity than SB-FI-26 to FABP5 and FABP7.

SB-FI-105 exhibited weak binding affinity to FABP5 with Ki value 3.93μM ±0.51, its binding affinity to FABP3 and FABP7 are even worse.SB-FI-401 was synthesized as diester, because of the its low solubility in DMSO, the Ki value of it can not be obtained.

SB-FI-86 was synthesized by Simon Tong, it exhibited low binding affinity to FABPs. Since SB-FI-86 doesn't have the free carboxylate which was thought to be essential for interaction affinity, it has low binding affinity for FABP3, FABP5, FABP7. Hence the monoester SB-FI-87 was synthesized and it will be sent to test Ki value.

1.5 Experimental

1.5.1 General Methods

^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker 300, 500 or 700 MHz NMR spectrometer. Chemical shifts(δ) are reported in ppm and the chemical shifts of solvent peaks were calibrated based on literature reported values. Melting points were measured on a Thomas-Hoover capillary melting point apparatus. High resolution mass spectrometry analysis was carried out on an Agilent LC-UV-TOF mass. TLC was performed on Sorbent Sorbent Technologies aluminum-backed Silica G TLC plates (Sorbent Technologies, 200μm, 20 cm × 20 cm), and column chromatography was conducted on silica gel 60 (Merck, 230 −400 mesh ASTM). Auto Dock and Autodock Vina were used to docking compounds to FABP5.

1.5.2 Materials

The chemicals were purchased from VWR International Company, Sigma-Aldrich Company, Fisher Scientific Company. Tetrohydrofuran was distilled from sodium and benzophenone under nitrogen. Dichloromethane was distilled from calcium hydride under nitrogen.

1.5.3 Experimental Procedure

α-Truxillic acid (1-1)

α-Cinnamic acid (2.00 g, 13.5 mmol) was placed in a pyrex dish, exposed to 350 nm UV light for 4 days with periodic shaking. After completion of the photoreaction, the light yellow solid was

washed with diethyl ether, after washing diethyl ether was removed by evaporator and solid was obtained. The solid was dissolved in ethanol to recrystallize to give α-truxillic acid (1.11 g, 55.6%). ^{1}H NMR (300 MHz, Acetone-d_6) δ 3.99 (dd, J = 10.0, 7.6 Hz, 2H), 4.44 (dd, J = 10.0, 7.6 Hz, 2H), 7.23 (t, J = 7.1 Hz, 2H), 7.33 (t, J = 7.4 Hz, 4H), 7.42 (d, J = 7.4 Hz, 4H). The proton NMR are consistent with the reported value. [16]

α-2,4-Bis(2-chlorophenyl)cyclobutane-1,3-dicarboxylic acid (1-2)

2-Chlorocinnamic acid (3.4 g, 18.4 mmol) was placed in a pyrex dish, exposed to 365 nm UV light for 14 days with periodic shaking. After completion of the photoreaction, the light yellow solid was washed with diethyl ether and ethyl acetate, after washing the solvent was removed by evaporator and solid was obtained. 3.0 g, yield 89%. m.p. 205 – 207 ℃; ^{1}H NMR (500 MHz, Acetone-d_6) δ 4.00 (dd, J = 4.2, 2.6 Hz, 2H), 4.90 (dd, J = 4.2, 2.6 Hz, 2H), 7.08-7.13 (m, 4H), 7.22 (m, 2H), 7.38 (m, 2H), 11.08 (s, 2H); ^{13}C NMR (125 MHz, Acetone-d_6) δ 41.9, 42.6, 126.5, 128.0, 128.2, 128.7, 128.9, 129.1, 134.2, 136.5, 172.7; HRMS (EIS) *m/z* calcd for $C_{18}H_{14}Cl_2O_4H^+$ 365.0342, found 365.0347 (Δ = -1.29 ppm).

α-2,4-Bis(2-methoxyphenyl)cyclobutane-1,3-dicarboxylic acid (1-3)

Trans-2-methoxycinnamic acid (1.28 g, 6.24 mmol) was placed in a pyrex dish, exposed to 365 nm UV light for 6 days with periodic shaking. After completion of the photoreaction, the light yellow solid was washed with diethyl ether and ethyl alcohol, after washing the solvent was removed by evaporator and solid was obtained (0.80 g, yield 62.5%). ^{1}H NMR (300 MHz, Acetone-d_6) δ 3.83 (s, 6H), 3.93-4.02 (m, 2H), 4.62 (dd, J = 10.1, 7.6 Hz, 2 H),6.94 (t, J = 7.4 Hz,

4H), 7.18 – 7.27 (m, 2H), 7.35 (d, *J* = 7.6 Hz, 2H), 10.50 (s, 2H). ^{13}C NMR (175 MHz, DMSO-d_6) δ 42.6, 43.9, 124.7, 127.1, 128.5, 129.2, 132.3, 137.8, 173.6. (^{13}C NMR was collected by Su)

α-2,4-Bis(2-bromophenyl)cyclobutane-1,3-dicarboxylic acid (1-4)

Trans-2-methoxycinnamic acid (2.0 g, 4.40 mmoles) was placed in a pyrex dish, exposed to 365 nm UV light for 6 days with periodic shaking. After completion of the photoreaction, the light yellow solid was washed with diethyl ether and ethyl alcohol, after washing the solvent was removed by evaporator and solid was obtained. 1.3 g, yield 67.0%. m.p. 217-220 ℃; ^{1}H NMR (300 MHz, Acetone-d_6) δ 3.96 (dd, J = 4.0, 2.3 Hz, 2H), 4.89 (dd, J = 4.0, 2.3 Hz, 2H), 7.11 – 6.98 (m, 2H), 7.21 (m, 2H), 7.40 (m, 4H), 10.98 (s, 2H). .^{13}C NMR (175 MHz, Acetone-d_6) δ 42.9, 44.5, 125.1, 127.1, 128.3, 18.9, 132.6, 138.2, 172.6; HRMS (EIS) *m/z* calcd for $C_{18}H_{14}Br_2O_4H^+$ 454.9312, found 454.9313 (Δ = -0.16 ppm)

α-2,4-Dicarboxycyclobutane-1,3-diyl)bis(pyridin-1-ium) chloride (2-5)

(*E*)-2-(2-carboxyvinyl)pyridin-1-ium chloride (755 mg, 2mmol) was grinded by a spatula into a powder. The powder was spread on a glass petri dish evenly, and exposed under a 365 nm UV lamp for 25 days with periodic shaking. The reaction was monitored by ^{1}H NMR. The mixture was purified using flash chromatography on silica gel with 10% methanol in dichloromethane to give the desired product, 566 mg, 75% yield, as brown solid. ^{1}H NMR (300 MHz, D_2O) δ 4.08 (dd, J = 10.6, 7.1 Hz, 2H), 4.58 (dd, J = 10.6, 7.1 Hz, 2H), 7.75 – 7.90 (m, 2H), 7.94 (m, 2H), 8.42 (m, 2H), 8.63 (m, 2H); HRMS (EIS) *m/z* calcd for $C_{16}H_{14}N_2O_4H^+$ 299.1026, found 299.1029 (Δ = -1.02 ppm).

(α-2,4-Diphenylcyclobutane-1,3-dicarboxylic acid mono-1-naphthyl ester) (4-1)

α-Truxillic acid (1.0 g, 3.4 mmol) was suspended in thionyl chloride (5.3 ml), and 4 drops of DMF was added to the suspension. The reaction mixture was heated to reflux for 3 h. The excess thionyl chloride and DMF was removed in vacuo and truxillic acyl chloride was obtained, which was used in the next reaction. To the solution of truxillic acyl chloride in THF (100 ml) was added 1-naphthol (0.4 mg, 2.9 mmol) and pyridine (1.6 ml, 20.3 mmol) was heated to reflux overnight. The reaction was quenched with addition of water (30ml), and stir the solution for 0.5 h. Ethyl acetate was add to the solution. The organic layer was washed with the addition of water and copper sulfate, the aqueous layer was separated. The organic layer was dried over MgSO4 and the solvent was removed by a rotary evaporator. The crude product was purified by flashed column chromatography on silica gel using ethyl acetate/hexane/acetic acid (25:74:1) as eluents to give SBFI26 as white solid (0.3 mg, 19.1%).^{1}H NMR (700 MHz, $CDCl_3$) δ 4.15 (dd, J = 10.6, 7.0 Hz, 1H), 4.40 (dd, J = 10.6, 7.0 Hz, 1H), 4.65 (m, 2H), 6.29 (m, 1H), 7.12 (m, 1H), 7.32 – 7.24 (m, 4H), 7.41 (m, 8H), 7.50 (m,2H), 7.63 (m, 1H), 7.77 (d, J = 8.2 Hz, 1H). (^{1}H NMR collected by Su) The proton NMR are consistent with the reported value. [13]

Di([1,1'-biphenyl]-3-yl) α-2,4-diphenylcyclobutane-1,3-dicarboxylate (4-3)

α-Truxillic acid (300 mg, 1.0 mmol) was suspended in thionyl chloride (5.0 ml), and 2 drops of DMF was added to the suspension. The reaction mixture was heated to reflux for 3 h. The excess thionyl chloride and DMF was removed in vacuo and truxillic acyl chloride was obtained, which was used in the next reaction. To the solution of truxillic acyl chloride in THF (15 ml) was added 1,1'-biphenyl-3-ol (170.2 mg, 0.8 mmol) and pyridine (7.5 ml), stirred at room temperature

overnight. The reaction was quenched with addition of water (30ml), and stir the solution for 0.5 h. Ethyl acetate was add to the solution. The organic layer was washed with the addition of water and copper sulfate, the aqueous layer was separated. The organic layer was dried over MgSO4 and the solvent was removed by a rotary evaporator. The crude product was purified by flashed column chromatography on silica gel using ethyl acetate/hexane/acetic acid (25:74:1) as eluents to give mono substituted ester and di-substituted ester. The di-substituted ester was separated as white solid (35 mg, 15%): mp 184-185 ℃; ^{1}H NMR (500 MHz, Acetone-d_6) δ 4.45 (dd, J = 10.8, 7.3 Hz, 2H), 4.81 (dd, J = 10.8, 7.3 Hz, 2H), 6.60 – 6.49 (m, 4H), 7.57 – 7.27 (m, 20H), 7.65 (d, J = 7.3 Hz, 4H); ^{13}C NMR (125 MHz, Acetone-d_6) δ 41.6, 46.4, 120.0, 120.5, 124.0, 126.9, 127.5, 127.7, 128.3, 128.6, 128.8, 129.5, 139.0, 139.7, 142.2, 151.2, 170.3;

α-2,4-Diphenyl-3-(tetrahydro-2H-pyran-4-ylmethoxycarbonyl)cyclobutane-1-carboxylic acid (4-4)

α-Truxillic acid (296 mg, 1.0 mmol) was suspended in thionyl chloride (5.0 ml), and 2 drops of DMF was added to the suspension. The reaction mixture was heated to reflux for 3 h. The excess thionyl chloride and DMF was removed in vacuo and truxillic acyl chloride was obtained, which was used in the next reaction. To the solution of truxillic acyl chloride in THF (15 ml) was added 2H-Pyran-4-methanol (348.5nmg, 3.0 mmol) and pyridine (7.5 ml), stirred at room temperature overnight. The reaction was quenched with addition of water (30ml), and stir the solution for 0.5 h. Ethyl acetate was add to the solution. The organic layer was washed with the addition of water and copper sulfate, the aqueous layer was separated. The organic layer was dried over MgSO4 and the solvent was removed by a rotary evaporator. The crude product was purified by flashed column

chromatography on silica gel using ethyl acetate/hexane/acetic acid (25:74:1) as eluents to give mono substituted ester and di-substituted ester. The mono-substituted ester was separated as white solid (38.4 mg). The di-substituted ester was separated as white solid (8.0 mg). ^{1}H NMR (300 MHz, Acetone-d_6) δ 0.87 – 1.12 (m, 2H), 1.25 (m, 2H), 1.37 – 1.58 (m, 1H), 3.17 (t, J = 11.6 Hz, 2H), 3.58 (d, J = 6.5 Hz, 2H), 3.75 (m, 2H), 3.99 (m, 7.3 Hz, 2H), 4.46 (m, 2H), 7.31 (m, 10H), 10.52 (s, 1H); ^{13}C NMR (175 MHz, Acetone-d_6) δ 34.3, 41.5, 41.7, 46.3, 46.8,66.8, 66.8, 68.4, 126.8,126.9, 127.7, 127.7, 128.2, 128.3, 139.5, 139.5, 171.5, 172.2.

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α-2,4-Bis(2-methoxyphenyl)-3-((quinolin-5-yloxy)carbonyl)cyclobutane-1-carboxylic acid (4-6)

α-2,4-Bis(2-methoxyphenyl)cyclobutane-1,3-dicarboxylic acid(366.4 mg, 1.0 mmol) was suspended in thionyl chloride (5.0 ml), and 2 drops of DMF was added to the suspension. The reaction mixture was heated to reflux for 3 h. The excess thionyl chloride and DMF was removed in vacuo and truxillic acyl chloride was obtained, which was used in next reaction. To the solution of truxillic acyl chloride in THF (15 ml) was added 5-quinolinol (116.1 mg, 0.8 mmol) and pyridine (7.5 ml), stirred at room temperature overnight. The reaction was quenched with addition of water (30ml), and stir the solution for 0.5 h. Ethyl acetate was add to the solution. The organic layer was washed with the addition of water and copper sulfate, the aqueous layer was separated. The organic layer was dried over $MgSO_4$ and the solvent was removed by a rotary evaporator. The crude product was purified by washed with ethyl acetate and methanol, and the desired product was obtained as off-white solid (115 mg, 29.7%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.81 (d, J =

2.5 Hz, 6H), 3.95 (dd, J = 10.4, 7.2 Hz, 1H), 4.40 (dd, J = 10.4, 7.2 Hz, 1H), 4.65 (dd, J = 10.4, 7.2 Hz, 1H), 4.74 (dd, J = 10.4, 7.2 Hz, 1H), 6.53 (m,1H), 7.03 – 6.97 (m, 2H), 7.06 (m,1H), 7.10 (m, 1H), 7.28 (m, 1H), 7.44 – 7.34 (m, 3H), 7.49 (m, 2H), 7.61 (m, 1H), 7.85 (m, 1H), 8.87 (m, 1H), 12.06 (s, 1H); ^{13}C NMR (175 MHz, DMSO-d_6) δ 36.6, 36.7, 40.5, 44.9, 45.3, 55.9, 56.1, 111.1, 111.4, 118.7, 120.6, 121.1, 122.1, 127.2, 127.50, 127.53, 127.8, 128.0, 128.6, 129.1, 129.4, 130.2, 146.4, 148.5, 151.4, 157.7, 157.9, 171.6, 173.5; HRMS (EIS) *m/z* calcd for $C_{29}H_{25}NO_6H^+$ 484.1755, found 484.1763 (Δ = -1.66 ppm).

α-2,4-Bis(2-methoxyphenyl)-3-(2-phenylcyclohexyloxy)carbonyl)cyclobutane-1-carboxylic acid (4-7)

α-2,4-Bis(2-methoxyphenyl)cyclobutane-1,3-dicarboxylic acid (183 mg, 0.5 mmol) was suspended in thionyl chloride (2.5 ml), and 2 drops of DMF was added to the suspension. The reaction mixture was heated to reflux for 3 h. The excess thionyl chloride and DMF was removed in vacuo and truxillic acyl chloride was obtained, which was used in the next reaction. To the solution of truxillic acyl chloride in THF (15 ml) was added (1*S*,2*S*)-2-phenylcyclohexan-1-ol (75 mg, 0.42 mmol) and pyridine (7.5 ml), stirred at room temperature overnight. The reaction was quenched with addition of water (30 ml), and stir the solution for 0.5 h. Ethyl acetate was add to the solution. The organic layer was washed with the addition of water and copper sulfate, the aqueous layer was separated. The organic layer was dried over $MgSO_4$ and the solvent was removed by a rotary evaporator. The crude product was purified by flashed column chromatography on silica gel using ethyl acetate/hexane/acetic acid (25:74:1) as eluents to give

desired product as off white solid (55.6mg, 27%). m.p. 163-165℃; ^{1}H NMR (500 MHz, Acetone-d_6) δ 0.74 – 0.83 (m, 1H), 1.19 – 1.37 (m, 2H), 1.52 (m, J = 12.6, 11.5, 2.8 Hz, 2H), 1.59 – 1.71 (m, 2H), 1.73 – 1.82 (m, 1H), 2.46 – 2.57 (m, 1H), 3.58 (dd, J = 10.2, 5.2 Hz, 1H), 3.69 (s, 3H), 3.79 (s, 3H), 3.92 – 4.03 (m, 2H), 4.41 (m, 1H), 4.66 (m 1H), 6.83 -7.33 (m, 13 H), 10.28 (s, 1H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 24.7, 25.9, 31.4, 37.3, 44.5, 49.7, 55.0, 55.2, 76.1, 110.0, 110.2, 120.2, 120.5, 126.5, 127.5, 128.0, 143.3, 157.4, 171.7, 178.6.(^{13}C NMR was collected by Su) HRMS (EIS) *m/z* calcd for $C_{32}H_{34}O_6H^+$ 515.2429, found 515.2426 (Δ = 0.42 ppm).

Chapter 2

Matrix metalloproteinase-9 inhibitors

2.1 Introduction

Matrix metalloproteinase(MMP) has been identified to play an important role in the propagation and spread of tumor cells.[17] Several series of compounds have been designed to bind with catalytic domains of MMPs and inhibit the catalytic activity of MMPs. However, the catalytic domains of all MMPs share a conserved binding site, the lack of specificity limited the development of MMP inhibitors as anti-cancer drugs.[18, 19]

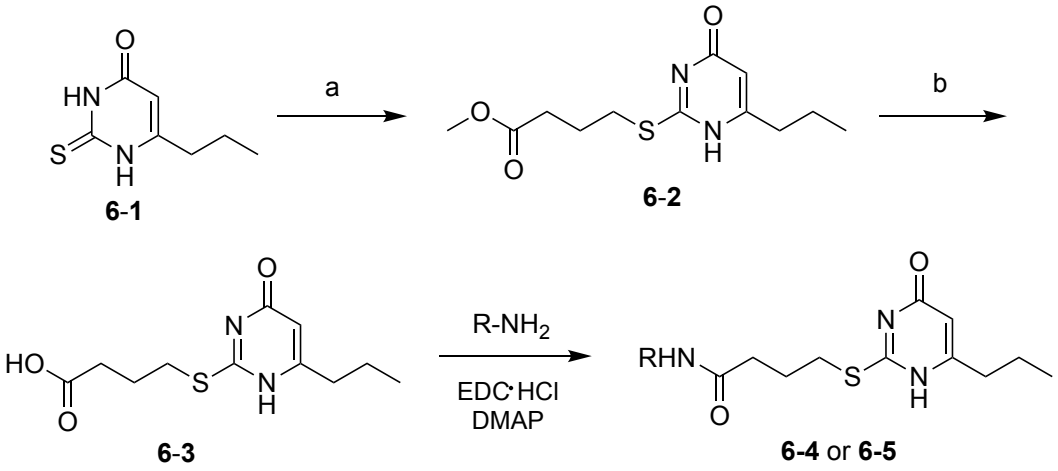
Detailed structural information about MMPs reveals that target less conserved, noncatalytic domains of the proteases would result in increasing target specificity and selectivity. Hemopexin (PEX), a noncatalytic site, can indirectly inhibits the activity of MMPs.[19-21] Since the PEX domains of MMPs are not conserved as highly as the catalytic sites, The PEX domain can be an alternative site to inhibit the biological roles of MMPs.[21]

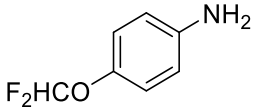
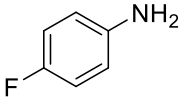
Previously, Tony Dufour and other coworkers used an in silico DOCKing approach to find small molecules that bind to the PEX domain of MMP-9. One of these compounds as lead compound *N*-[4-(difluoromethoxy)phenyl]-2-[(4-oxo-6-propyl-1*H*-pyrimidin-2-yl)sulfanyl]-acetamide, expressed micromolar affinity for MMP-9 with good selectivity, it inhibited MMP-9 mediated and induced cell migration.[22] Then Kunal and coworkers generated an library of derivatives of the lead compound to find more potent compound. After screening by DOCKing, the top 14 compounds with the highest binding affinitywere synthesized and sent to test, and they found one of these compounds exhibited better inhibition than the lead compound. These fifteen compound were re-synthesized to obtain the characterization data by Xiaodong and Qianwen.

2.2 Results and Discussion

Synthesis of compound **6-4** and **6-5** (Scheme 1) started from commercially available 6-n-propyl-2-thiouracil (**6-1**), which was alkylated by methyl 4-bromobutanoate under basic condition to gave methyl 4-((4-oxo-6-propyl-1,4-dihydropyrimidin-2-yl)thio)butanoate (**6-2**) in yield of 47%. Counpund **6-2** was hydrolyzed to corresponding 4-((4-oxo-6-propyl-1,4-dihydropyrimidin-2-yl)thio)butanoic acid (**6-3**) as a common intermediate by LiOH in yield of 66%. Compound **6-3** reacted with different amines to give compound **6-4** and **6-5** by EDC·HCl promoted coupling.

Scheme 6. Synthesis of compound **6-4** and **6-5**

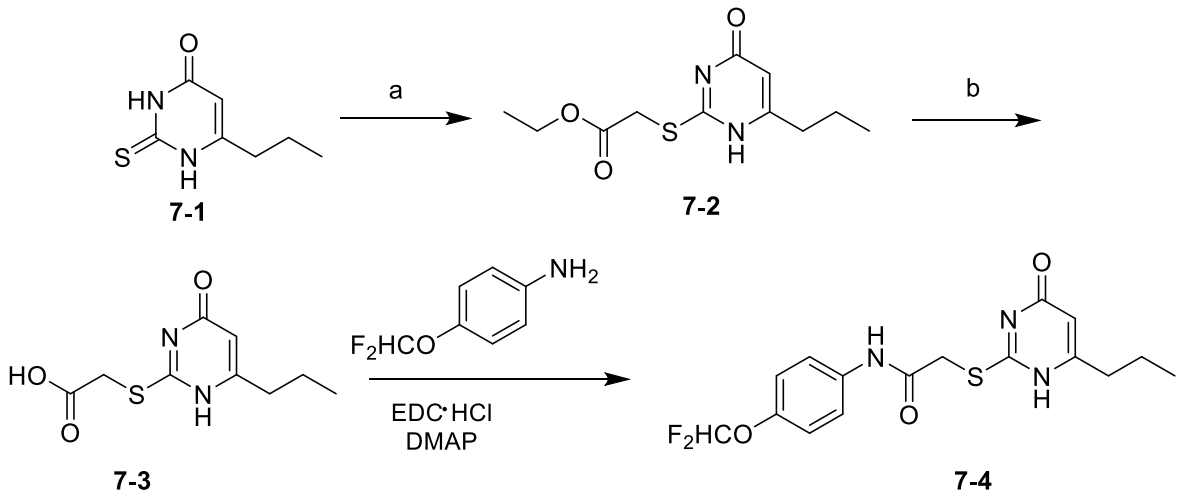


Compound Number	R-NH_2	Yield
6-4		41%
6-5		39%

Reagents and conditions: (a) Methyl 4-bromobutanoate, K_2CO_3, MeOH, H_2O, reflux, 1h, 47%; (b) LiOH, MeOH, H_2O, RT, 2 days, 66%.

Synthesis of compound A (Scheme 2) started from commercially available 6-n-propyl-2-thiouracil (**7-1**), which was alkylated by ethyl 2-bromoacetate under basic condition gave ethyl 2-((4-oxo-6-propyl-1,4-dihydropyrimidin-2-yl)thio)acetate (**7-2**) in yield of 67%. Counpund **7-2** was hydrolyzed to corresponding 4-((4-oxo-6-propyl-1,4-dihydropyrimidin-2-yl)thio)butanoic acid (2-3) as a common intermediate by LiOH in yield of 32%. Compound **7-3** reacted with 4-(difluoromethoxy)aniline to give compound **7-4** by EDC·HCl promoted coupling in yield of 8%.

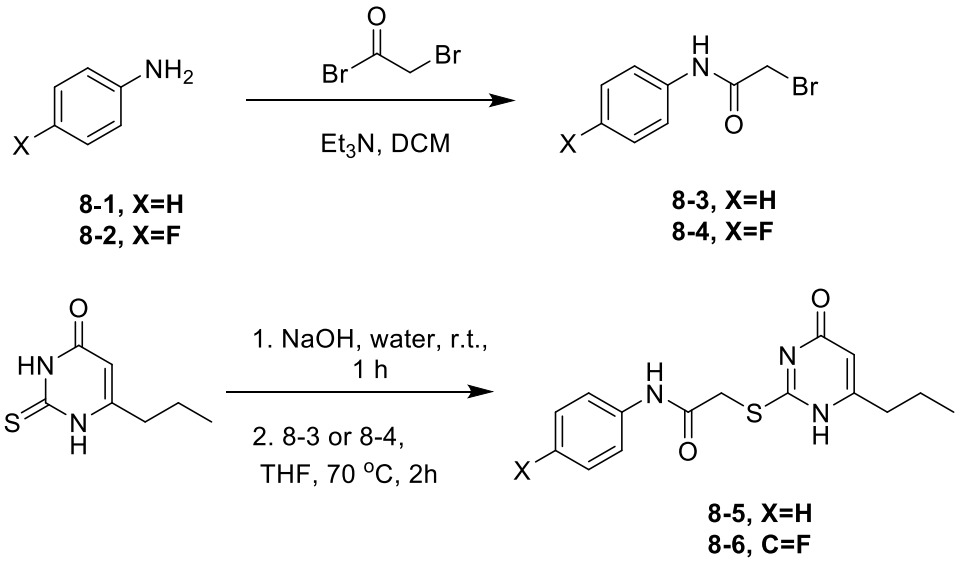
Scheme 7. Synthesis of compound **7-4**

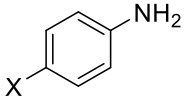
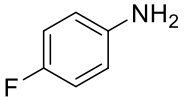
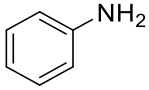


Reagents and conditions: (a) Ethyl 2-bromoacetate, K_2CO_3, MeOH, H_2O, r.t., 15 min, 67%; (b) LiOH, MeOH, H_2O, RT, 2 days, 32%.

Synthesis of compound **8-5** and **8-6** (Scheme 3) started with the coupling of amine **8-1-2** with 2-bromoacetyl bromide to afford compound **8-3-4**, in the yield of 28% and 41% respectively. Subsequently, it was treated with the solution of 6-n-propyl-2-thiouracil in aqueous NaOH and heated to 70 ℃for 2 hours to form the desired product compound **8-5** and **8-6** as white solid.

Scheme 8. Synthesis of compound **8-5** and **8-6**



Compound Number		Yield
8-5		69 %
8-6		35 %

2.3 Summary

Compounds **6-4**, **6-5**, **7-4**, **8-5**, **8-6** were synthesized and fully characterized.

2.4 Experimental

2.4.1 General Methods

^{1}H, ^{13}C and NMR spectra were measured on a Bruker 500, or 700 MHz spectrometer. Melting points were measured on a Thomas-Hoover capillary melting point apparatus. TLC was performed on Sorbent Technologies aluminum-backed Silica G TLC plates (Sorbent Technologies, 200μm, 20 cm × 20 cm), and column chromatography was carried out on silica gel 60 (Merck, 230 −400 mesh ASTM). High-resolution mass spectrometry analysis was carried out on an Agilent LC −UV−TOF mass spectrometer.

2.4.2 Materials

The chemicals were purchased from Sigma-Aldrich, Fisher Scientific, and VWR International and used as received or purified before use by standard methods. Tetrahydrofuran was freshly distilled from sodium and benzophenone. Dichloromethane was also distilled immediately prior to use under nitrogen from calcium hydride.

2.4.3 Experimental procedure

Methyl 4-(4-oxo-6-propyl-1,4-dihydropyrimidin-2-ylthio)butanoate (6-2)

To a solution of 6-n-propyl-2-thiouracil (0.85 g, 5 mmole) and potassium carbonate (1.03g, 7.5 mmole) in MeOH (5 mL) and water (10 mL) was added methyl 4-bromobutanoate (1.36g, 7.5 mmol). The solution was heated to reflux overnight. After the completion of reaction, the mixture was cooled to RT, 50 ml water was added, and the mixture was extracted with ethyl acetate (3 × 50 mL). The organic layers were combined and washed with water (50 mL) and brine (50 mL),

dried over $MgSO_4$, the solvent was removed by rotary evaporator. The crude product was purified by flash column chromatography on silica gel using MeOH/DCM to give desired product as white solid (0.64 g, 47%): mp. 129-130 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.95 (t, *J* = 7.4 Hz, 3H), 1.71 – 1.64 (m, 2H), 2.06 (m, 2H), 2.46 (m, 4H), 3.24 (t, *J* = 7.0 Hz, 2H) , 3.68 (s, 3H) , 6.04 (s, 1H), 12.99 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.8, 21.0, 24.7, 29.9, 32.7, 39.7, 51.8, 108.0, 160.2, 165.6, 169.5, 173.4. HRMS (EIS) *m/z* calcd for $C_{12}H_{18}N_2O_3SH^+$ 271.111, found 271.111 (Δ = 0.51 ppm).

4-(4-Oxo-6-propyl-1,4-dihydropyrimidin-2-ylthio)butanoic acid (6-3)

To a solution of methyl 4-(4-oxo-6-propyl-1,4-dihydropyrimidin-2-ylthio)butanoate (500 mg, 1.84 mmol) in MeOH and water was added lithium hydroxide (88.1 mg, 3.68 mmol), and the mixture was stirred at RT for 2 days. After the completion of the reaction, the mixture was adjusted to pH 1 by 1N hydrochloric acid solution, and white precipitate was observed. The precipitate was filtered to afford desired product as white solid (308.0 mg, 66%): mp. 139-141 ℃; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (t, *J* = 7.3 Hz, 3H), 1.68 (m, 2H), 2.14 – 2.01 (m, 2H), 2.49 (m, 4H), 3.28 (t, *J* = 6.5 Hz, 2H), 6.04 (s, 1H). ^{13}C NMR (175 MHz, $CDCl_3$) δ 13.8, 21.1, 24.5, 29.9, 32.8, 39.7, 107.7, 160.3, 165.9, 170.2, 178.5. HRMS (EIS) *m/z* calcd for $C_{11}H_{16}N_2O_3SH^+$ 257.0954, found 257.0958 (Δ = 1.33 ppm).

***N*-(4-Difluoromethoxyphenyl)-4-(4-oxo-6-propyl-1,4-dihydropyrimidin-2-ylthio)butanamide (6-4)**

To a mixture of 4-(4-oxo-6-propyl-1,4-dihydropyrimidin-2-ylthio)butanoic acid (190 mg, 0.74 mmol), 4-(difluoromethoxy)aniline (130.5 mg, 0.82 mmol) and 4-(dimethylamino)pyridine (99.4

mg, 0.81 mmol) in DMF (3 mL) was added N'-ethylcarbodiimide hydrochloride (156 mg, 0.81 mmol), the mixture was stirred at room temperature for 1 hour. After the reaction completion, 100 mL water was added to it, and off white precipitate was observed which was filtered to give off-white solid. The solid was dissolved in ethyl acetate and dried over $MgSO_4$ and filtered, the solvent was removed by rotary evaporator. The crude product was purified by crystallizing from ethyl acetate and hexane to afford desired compound as white solid (120.4 mg, 41%): mp. 174-177 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.92 (t, *J* = 7.3 Hz, 3H), 1.64 (m, 2H), 2.06 – 2.22 (m, 2H), 2.42 (t, *J* = 7.4 Hz, 2H) , 2.49 (t, *J* = 6.9 Hz, 2H), 3.31 (t, *J* = 5.7 Hz, 2H), 6.01 (s, 1H), 6.45 (t, *J* = 74.0 Hz, 1H), 7.07 (d, *J* = 8.6 Hz, 2H), 7.55 (d, *J* = 8.6 Hz, 2H), 7.92 (s, 1H), 12.69 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.8, 21.1, 24.8, 30.2, 35.5, 39.7, 107.8, 116.1(t, *J* = 259 Hz), 120.6, 121.3, 135.6, 147.3, 160.2, 165.5, 169.9, 170.5. HRMS (EIS) *m/z* calcd for $C_{18}H_{21}F_2N_3O_3SH^+$398.1344, found 398.1345 (Δ = -0.03 ppm).

_N_-(4-Fluorophenyl)-4-(4-oxo-6-propyl-1,4-dihydropyrimidin-2-ylthio)butanamide (6-5)

To a mixture of 4-(4-oxo-6-propyl-1,4-dihydropyrimidin-2-ylthio)butanoic acid (175.6 mg, 0.67 mmol), 4-fluoroaniline (83.3 mg, 0.75 mmol) and 4-(dimethylamino)pyridine (91.6 mg, 0.75 mmol) in DMF (3 mL) was added *N'*-ethylcarbodiimide hydrochloride (142.6 mg, 0.75 mmol). The mixture was stirred at room temperature for 1 hour. After the reaction completion, 100 mL water was added to it, and off white precipitate was observed which was filtered to give off-white solid. The solid was dissolved in ethyl acetate and dried over $MgSO_4$ and filtered, the solvent was removed by rotary evaporator. The crude product was purified by crystallizing from ethyl acetate and hexane to afford desired compound as white solid (90.8 mg, 39%): m.p. 180-182 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.96 – 0.83 (t, *J* = 7.3 Hz, 3H), 1.69 – 1.57 (m, 2H), 2.21 – 2.05 (m, 2H),

2.55 – 2.33 (m, 4H), 3.31 ((t, J = 6.3 Hz, 2H), 6.02 (s, 1H), 7.00 (t, J = 8.7 Hz, 2H), 7.49 (m, 2H), 7.64 (s, 1H), 12.45 (s, 1H). ^{13}C NMR (175 MHz, DMSO-d_6) δ 13.9, 20.9, 25.3, 29.7, 35.3,39.0, 115.6 (d, J = 22.0Hz), 121.2 (d, J = 7.7Hz), 136.1 (d, J = 2.3Hz), 157.6, 159.0, 170.7. HRMS (EIS) *m/z* calcd for $C_{17}H_{20}FN_3O_2SH^+$ 350.1333, found 350.1340 (Δ = -2.12 ppm).

Ethyl 2-(4-oxo-6-propyl-1,4-dihydropyrimidin-2-ylthio)acetate (7-2)

To a solution of 6-n-propyl-2-thiouracil (1.70 g, 10.0 mmol) and potassium carbonate (2.07 g, 15.0 mmol) in MeOH (10 mL) and water (20 mL) was added ethyl 2-bromoacetate (1.42 g, 8.5 mmol). The mixture was stirred at RT for 15 min. After the completion of reaction, 50 ml water was added to it. Then the mixture was extracted with ethyl acetate (5 × 80 mL). The organic layers were combined and washed with water (100 mL) and brine (100 mL), dried over $MgSO_4$, the solvent was removed by rotary evaporator. The crude product was purified by flash column chromatography on silica gel using MeOH/DCM to give desired product as white solid (1.7 g, 67%): mp. 117-118 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.93 (t, J = 7.4 Hz, 3H), 1.27 (t, J = 7.1 Hz, 3H), 1.59 – 1749 (m, 2H), 2.43 (t, J = 7.5 Hz, 2H), 3.93 (s, 2H) , 4.20 (m, 2H), 6.05 (s, 1H), 13.18 (s, 1H). ^{13}C NMR (175 MHz, $CDCl_3$) δ 13.6, 14.1, 20.8, 32.8, 39.5, 61.9, 108.2, 158.9, 165.5, 168.2, 169.2. HRMS (EIS) *m/z* calcd for $C_{11}H_{16}N_2O_3SH^+$ 257.0954, found 257.0953 (Δ = 0.49 ppm).

2-(4-Oxo-6-propyl-1,4-dihydropyrimidin-2-ylthio)acetic acid (7-3)

To a solution of ethyl 2-((4-oxo-6-propyl-1,4-dihydropyrimidin-2-yl)thio)acetate (1.2g, 4.68 mmol) in MeOH and water was added Lithium hydroxide (0.2 g, 9.36 mmol). The mixture was stirred at r.t. for 2 days. After the completion of the reaction, the mixture was adjusted to pH=1 by

1N hydrochloric acid solution, and extracted with ethyl acetate (5×80 mL). The organic layers were combined and dried over MgSO4, and the solvent was removed by rotary evaporator to afford desired product as white solid (0.3 g, 28%): m.p. 154-155 ℃; ^{1}H NMR (700 MHz, DMSO-d_6) δ 0.86 (t, J = 7.2 Hz, 3H), 1.56 (m, 2H), 2.30 (t, J = 7.1 Hz, 2H), 3.47 (s, 2H), 5.79 (s, 1H). ^{13}C NMR (175 MHz, DMSO-d_6) δ 14.0, 21.1, 36.5, 38.7. HRMS (EIS) *m/z* calcd for $C_9H_{13}N_2O_3SH^+$ 229.0641, found 229.0644 (Δ = -1.11 ppm).

_N_-(4-(difluoromethoxy)phenyl)-2-((4-oxo-6-propyl-1,4-dihydropyrimidin-2-yl)thio)acetamide (7-4)

To a mixture of 2-((4-Oxo-6-propyl-1,4-dihydropyrimidin-2-yl)thio)acetic acid (137.5 mg, 0.6 mmol), 4-(difluoromethoxy)aniline (105.0 mg, 0.7 mmol) and 4-(dimethylamino)pyridine (80.6 mg, 0.7 mmol) in DMF (3 mL) was added *N'*-ethylcarbodiimide hydrochloride (125.5mg, 0.7 mmol). The mixture was stirred at room temperature for 1 hour. After the reaction completion, 100 mL water was added to it, and off white precipitate was observed which was filtered to afford desire product (18 mg, 8 %): m.p.: decomposed at 180 ℃; ^{1}H NMR (700 MHz, DMSO-d_6) δ 0.73 (t, J = 7.2 Hz, 3H), 1.51 (m, 2H), 2.32 (t, J = 7.1 Hz, 2H), 4.05 (s, 2H), 5.97 (s, 1H) ,6.97 – 7.29 (m, 3H), 7.62 (d, J = 8.4 Hz, 2H), 10.44 (s, 1H), ^{13}C NMR (175 MHz, DMSO-d_6) δ 13.9, 21.0, 35.5, 38.7, 117.0 (t, J = 256.0 Hz), 112.0, 121.0, 136.9, 146.7, 166.3. HRMS (EIS) *m/z* calcd for $C_{16}H_{17}F_2N_3O_3SH^+$ 370.1031, found 370.1039 (Δ = 2.02 ppm).

2-Bromo-N-phenylacetamide (8-3)

To a solution of aniline (102 mg, 1.10 mmole) and triethylamine (223 mg, 2.20 mmole) in dichloromethane, bromoacetyl bromide (222 mg, 1.10 mmole) was added dropwise and stirred at 0 ℃ for 25 min. The reaction was monitored by TLC. After the completion of the reaction, the organic layer was washed with saturated NH_4Cl solution for 3 times, the aqueous layer was separated. The organic layer was dried over $MgSO_4$ and the solvent was removed by rotary evaporator. The crude product was purified by flashed column chromatography on silica gel using ethyl acetate/hexane to give desired product as white solid (65 mg, 28%): mp 134-136 ^{o}C (lit.[23] 134-135 °C). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.02 (s, 2H), 7.17 (m, 1H), 7.36 (t, J = 8.0 Hz, 2H), 7.53 (d, J = 8.0 Hz, 2H), 8.14 (s,1H). ^{13}C NMR (175 MHz, $CDCl_3$) δ 29.5, 120.0, 125.2, 129.2, 136.9, 163.3. These data are consistent with the reported value.[24]

2-Bromo-*N*-(4-fluorophenyl)acetamide (8-4)

To a solution of 4-fluoroaniline (244 mg, 2.2 mmole) and triethylamine (446 mg, 4.4 mmole) in dichloromethane, bromoacetyl bromide (444 mg, 2.2 mmole) was added dropwise and stirred at 0 ℃ for 20 min. The reaction was monitored by TLC. After the completion of the reaction, the organic layer was washed with saturated NH_4Cl solution for 3 times, the aqueous layer was separated. The organic layer were combined and dried over $MgSO_4$ and the solvent was removed by rotary evaporator. The crude product was purified by flash column chromatography on silica gel using ethyl acetate/hexane to give desired product as white solid (207 mg, 41%): mp. 133-135 ℃ （lit[25] 133-136 ℃）; ^{1}H NMR (500 MHz, Acetone-d_6) δ 4.03 (s, 2H), 7.05 – 7.13 (m, 2H), 7.65 – 7.72 (m, 2H), 9.58 (s, 1H). The proton NMR are consistent with the reported value.[26]

2-(4-Oxo-6-propyl-1,4-dihydropyrimidin-2-ylthio)-*N*-phenylacetamide (8-5)

To a solution of sodium hydroxide (7.2 mg, 0.18 mmol) in water (3 mL), 6-n-propyl-2-thiouracil was added (30 mg, 0.18 mmol) and stirred for 15 min. To the reaction mixture was added the solution of 2-bromo-N-phenylacetamide (38.5 mg, 0.18 mmol) in THF (3 mL) and heated at 70 ^{o}C for 48 h. After the completion of reaction white precipitate was observed and filtered. The crude product was purified by crystallizing from chloroform to afford desired compound as white solid (20mg, 35%): m.p. 170 ℃(decomposed); ^{1}H NMR (700 MHz, DMSO-d_6) δ ^{1}H NMR (700 MHz, DMSO-d_6) δ 0.83 (t, J = 7.0Hz, 3H), 1.46 - 1.56 (m, 2H), 2.31 (t, J = 7.0Hz, 2H), 4.04 (s, 2H), 5.90 (s, 1H), 7.04 (t, J = 7.7Hz, 1H), 7.30 (t, J = 7.7Hz, 2H), 7.56 (t, J = 7.7Hz, 2H), 10.33 (s, 1H), 12.67 (s, 1H). ^{13}C NMR (175 MHz, DMSO-d_6) δ 13.4, 20.5, 35.1, 38.5, 119.0, 123.3, 128.7, 139.0, 165.9. HRMS (EIS) *m/z* calcd for $C_{15}H_{17}N_3O_2SH^+$ 304.1114, found 304.1113 (Δ = 0.29 ppm).

***N*-(4-Fluorophenyl)-2-(4-oxo-6-propyl-1,4-dihydropyrimidin-2-ylthio)acetamide (8-6)**

To a solution of sodium hydroxide (7.2 mg, 0.18 mmol) in water (3 mL), 6-n-propyl-2-thiouracil was added (30 mg, 0.18 mmol) and stirred for 15 min. To the reaction mixture was added the solution of 2-bromo-N-phenylacetamide (38.5 mg, 0.18 mmol) in THF (3 mL) and heated at 70 ^{o}C for 48 h. After the completion of reaction white precipitate was observed and filtered. The crude product was purified by crystallizing from chloroform to afford desired compound as white solid (20mg, 35%): m.p. 170℃ (decomposed); ^{1}H NMR (700 MHz, DMSO-d_6) δ ^{1}H NMR (700 MHz, DMSO-d_6) δ 0.83 (t, J = 7.0Hz, 3H), 1.46 - 1.56 (m, 2H), 2.31 (t, J = 7.0Hz, 2H), 4.04 (s, 2H), 5.90 (s, 1H), 7.04 (t, J = 7.7Hz, 1H), 7.30 (t, J = 7.7Hz, 2H), 7.56 (t, J = 7.7Hz, 2H), 10.33 (s, 1H),

12.67 (s, 1H). ^{13}C NMR (175 MHz, DMSO-d_6) δ 13.4, 20.5, 35.1, 38.5, 119.0, 123.3, 128.7, 139.0, 165.9. HRMS (EIS) *m/z* calcd for $C_{15}H_{16}FN_3O_2SH^+$ 322.1020, found 322.1020 (Δ = -0.03 ppm).

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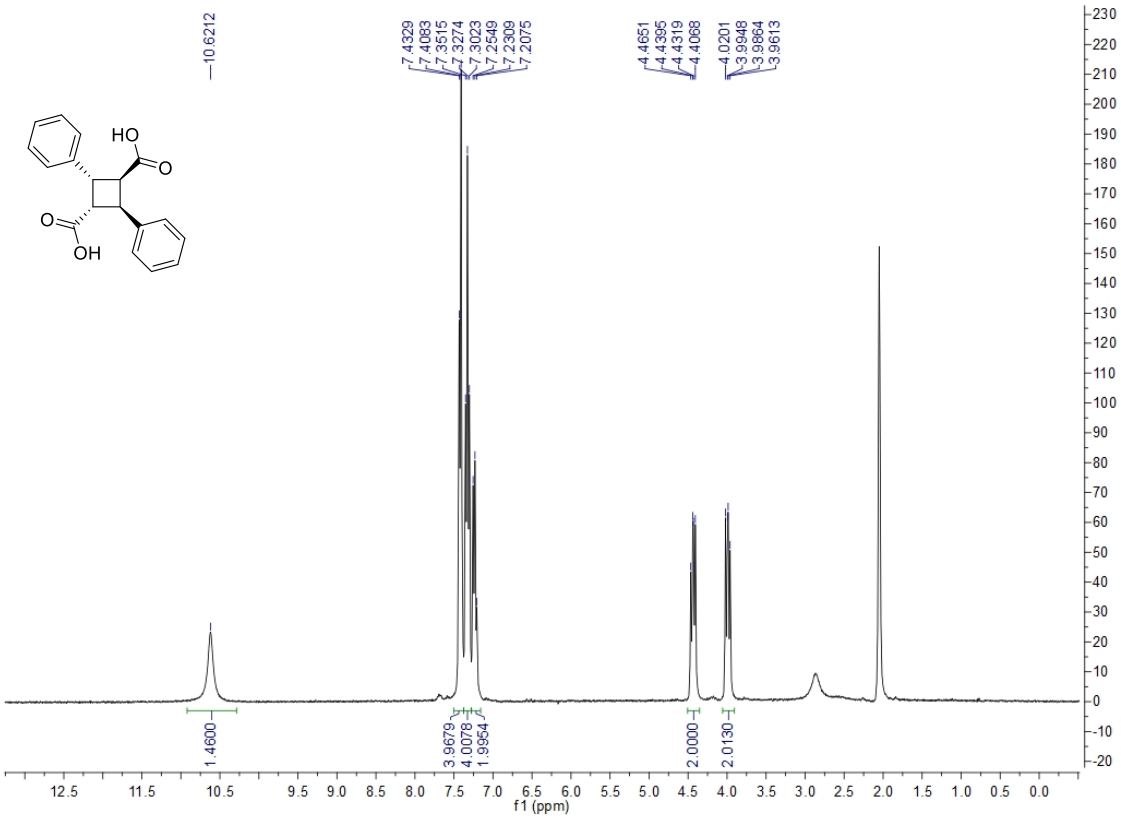
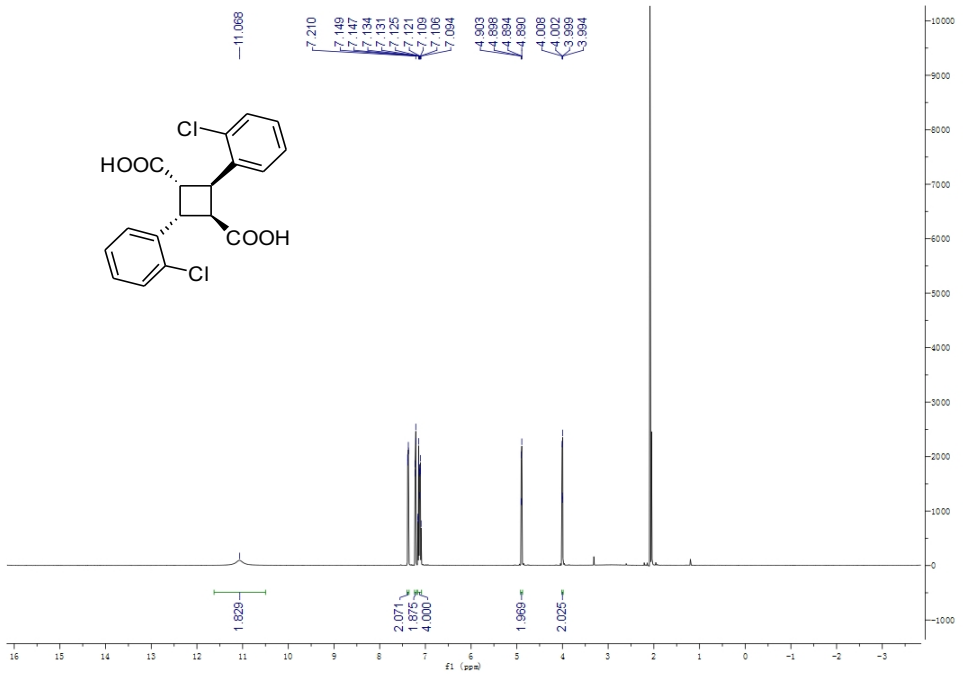
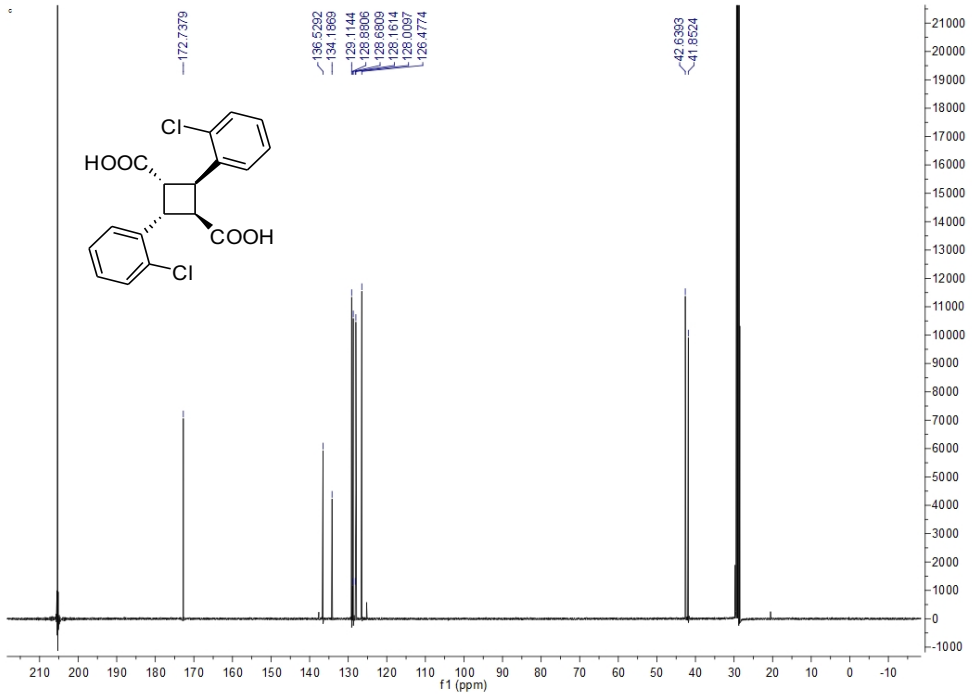
Appendix

α-Truxillic acid (1-1)



α-2,4-Bis(2-chlorophenyl)cyclobutane-1,3-dicarboxylic acid (1-2)





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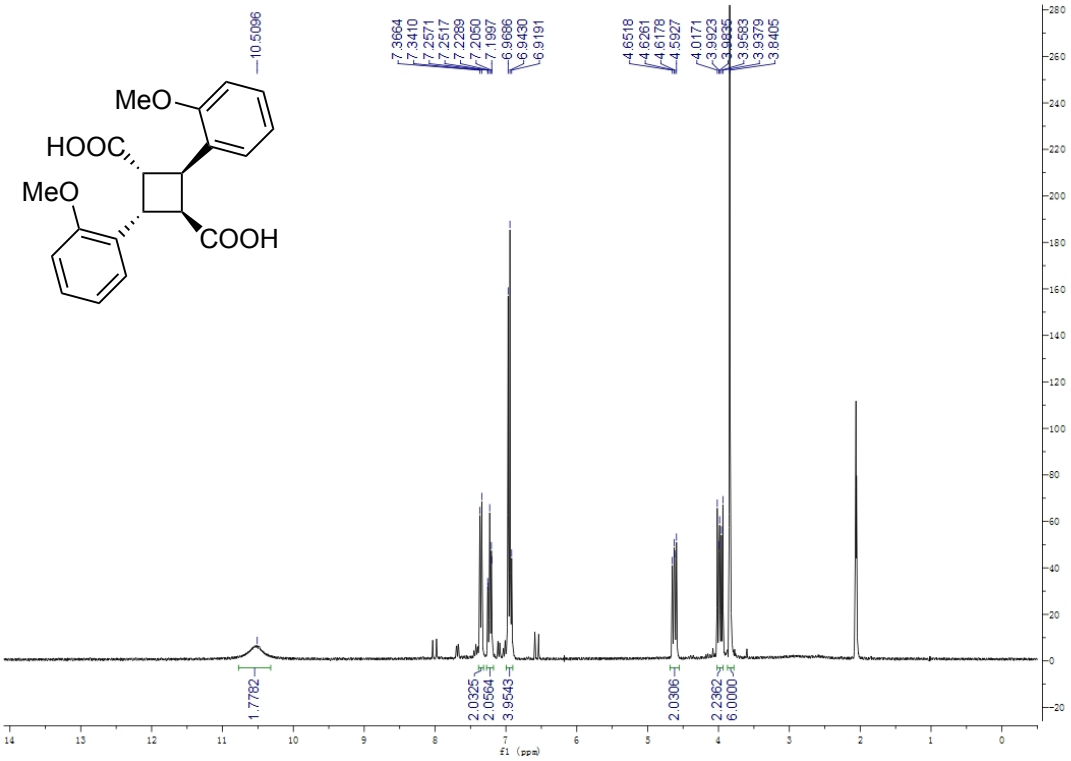
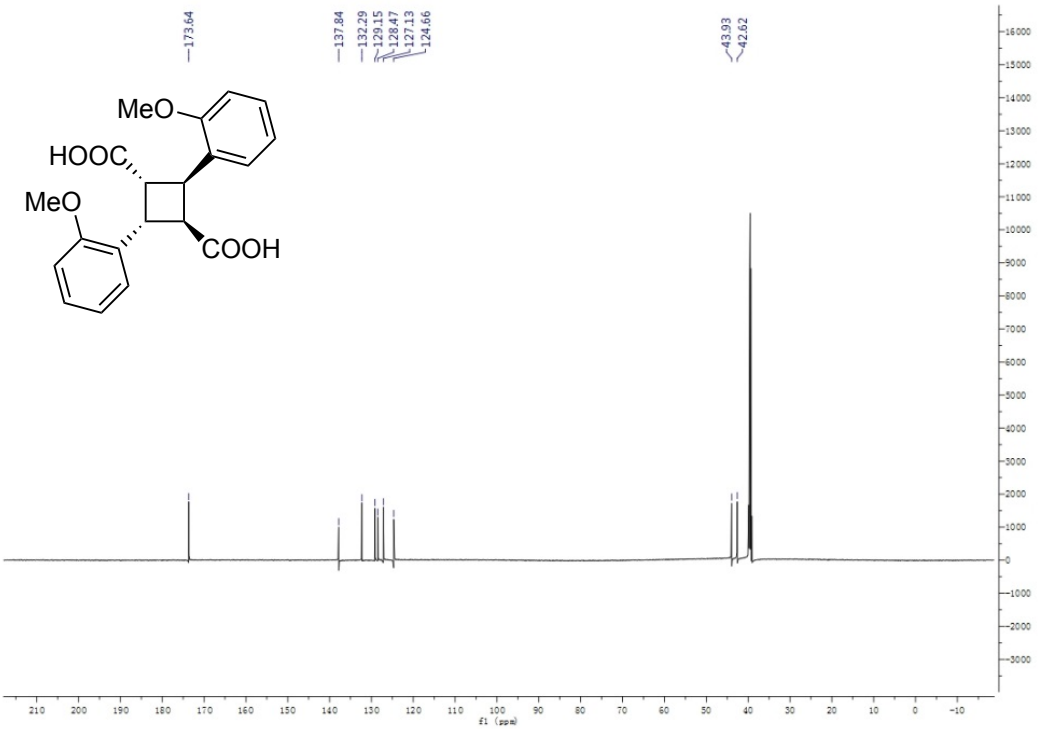
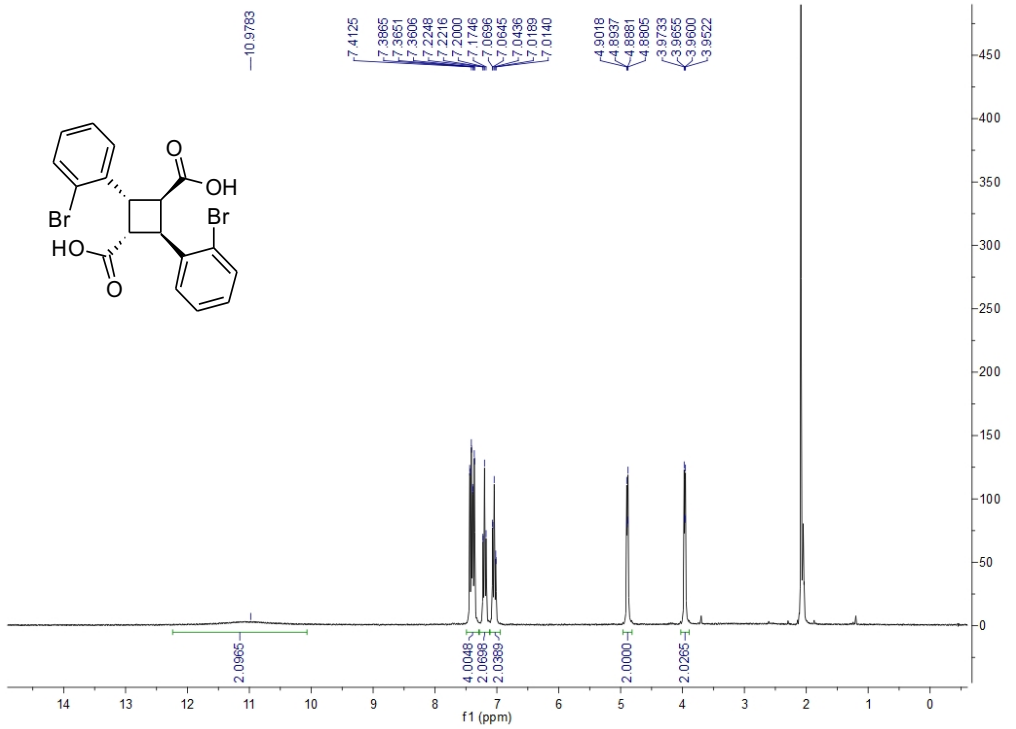
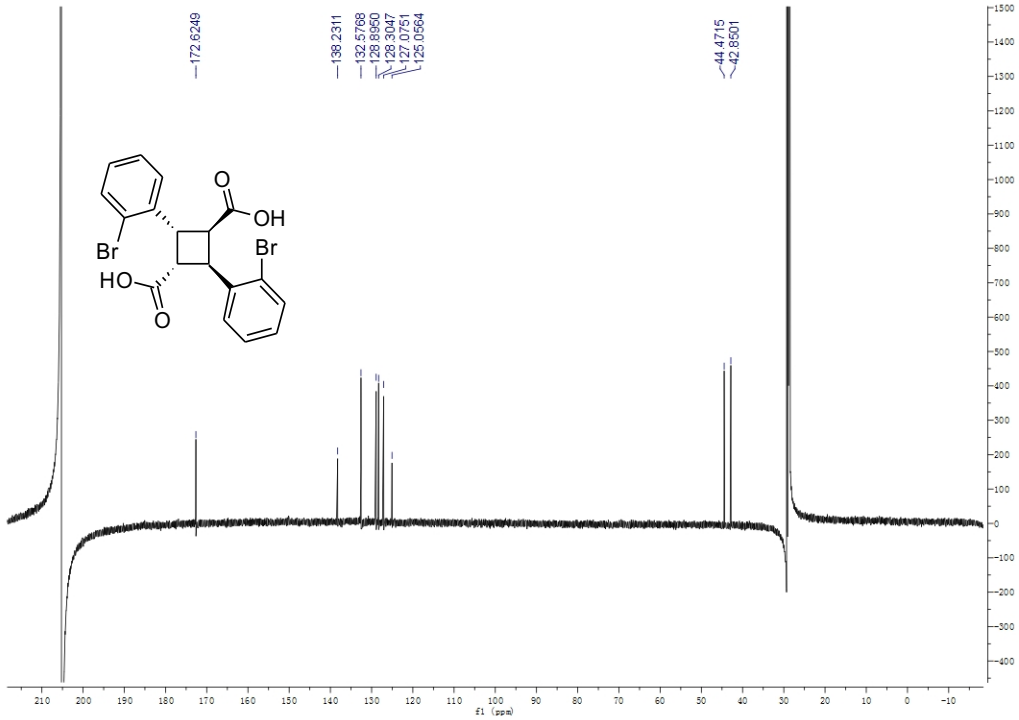


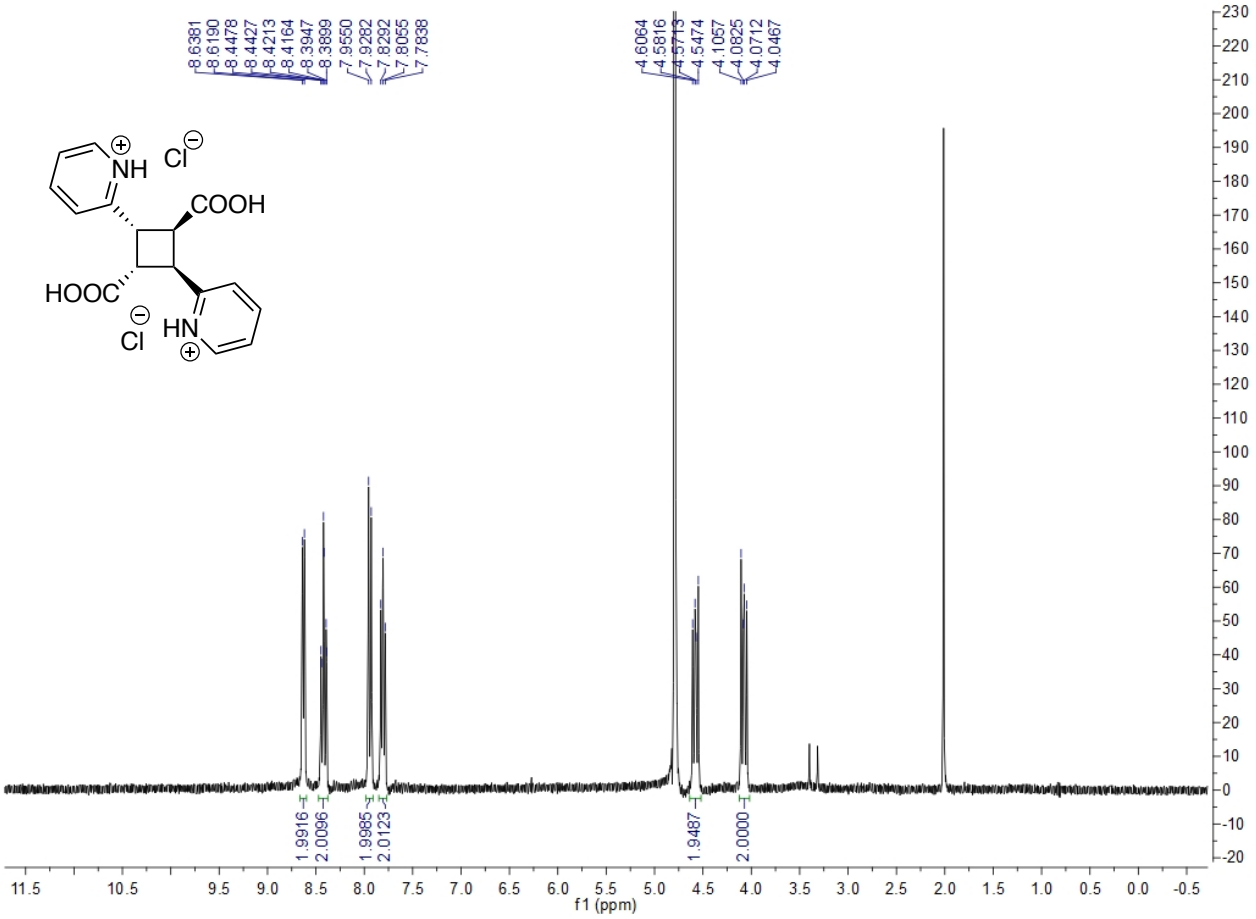


α-2,4-Bis(2-bromophenyl)cyclobutane-1,3-dicarboxylic acid (1-4)

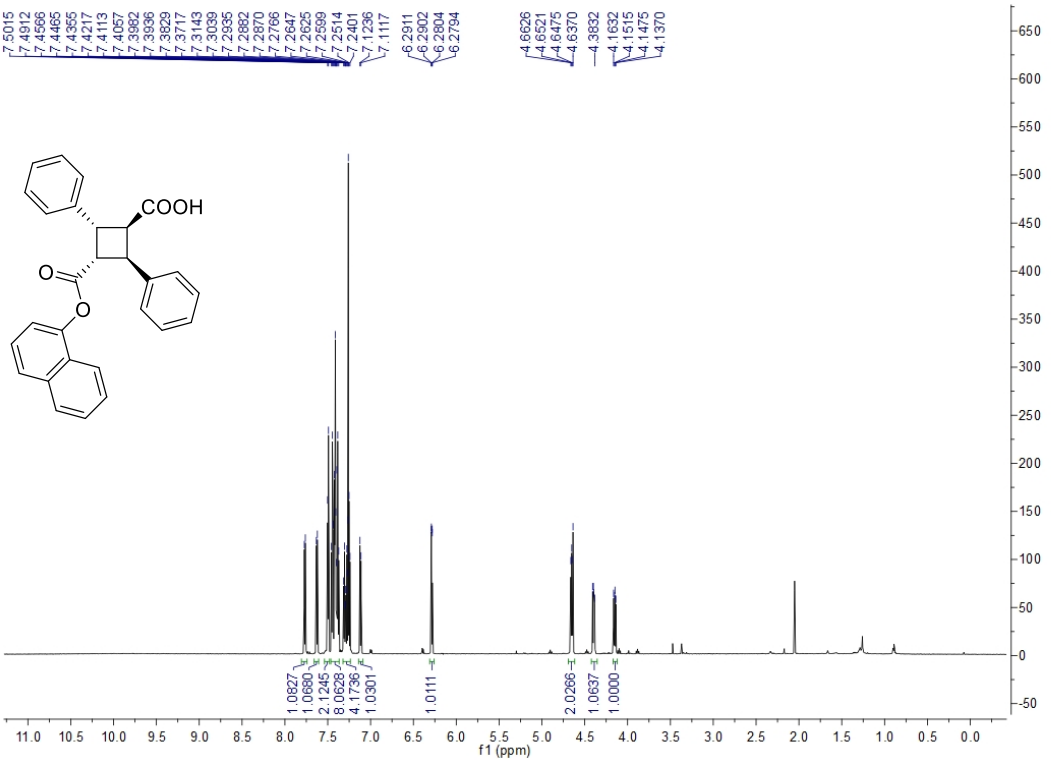




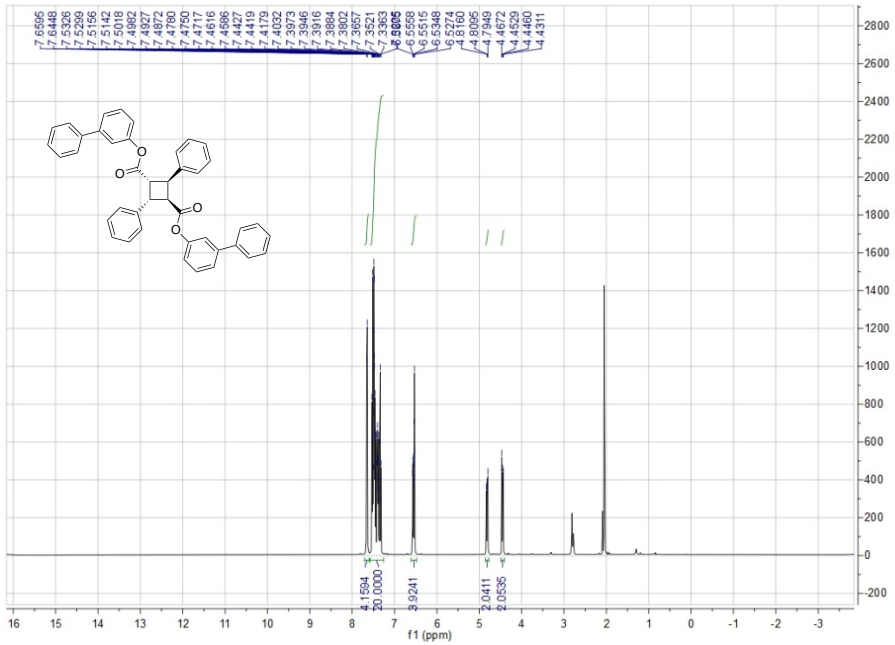
α-2,4-dicarboxycyclobutane-1,3-diyl)bis(pyridin-1-ium) chloride (2-5)

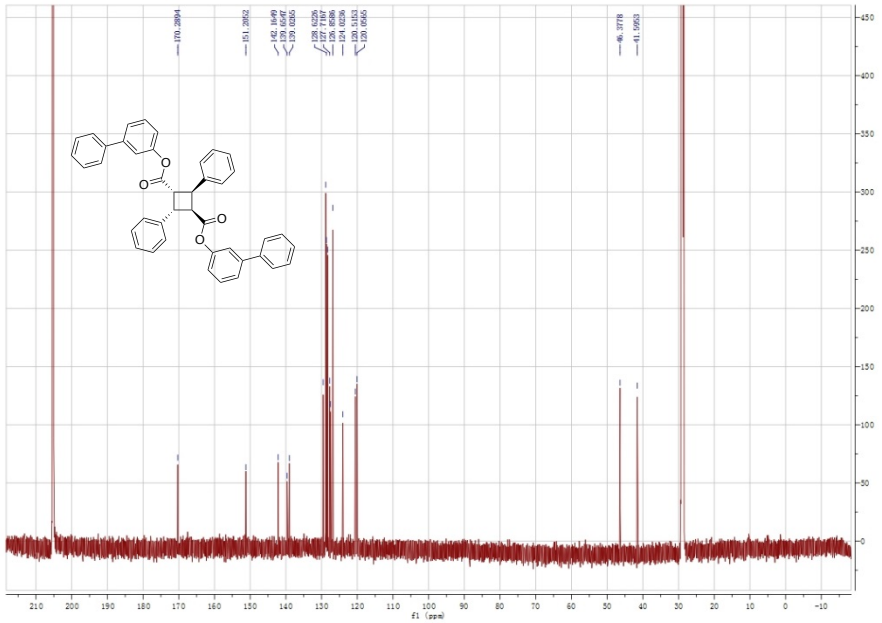


(α-2,4-Diphenylcyclobutane-1,3-dicarboxylic acid mono-1-naphthyl ester) (4-1)

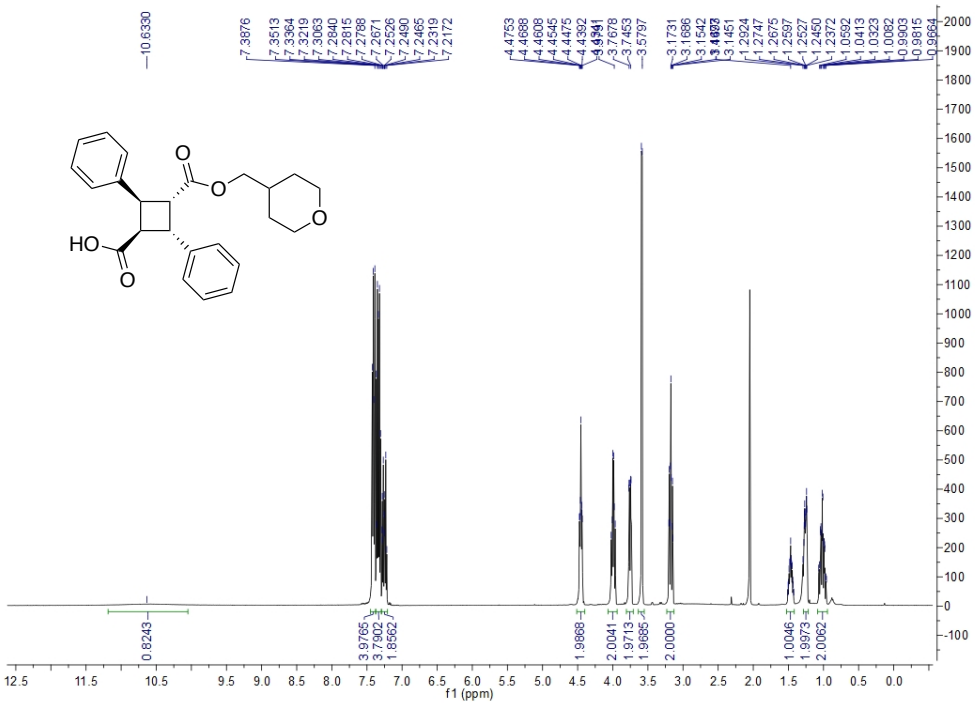


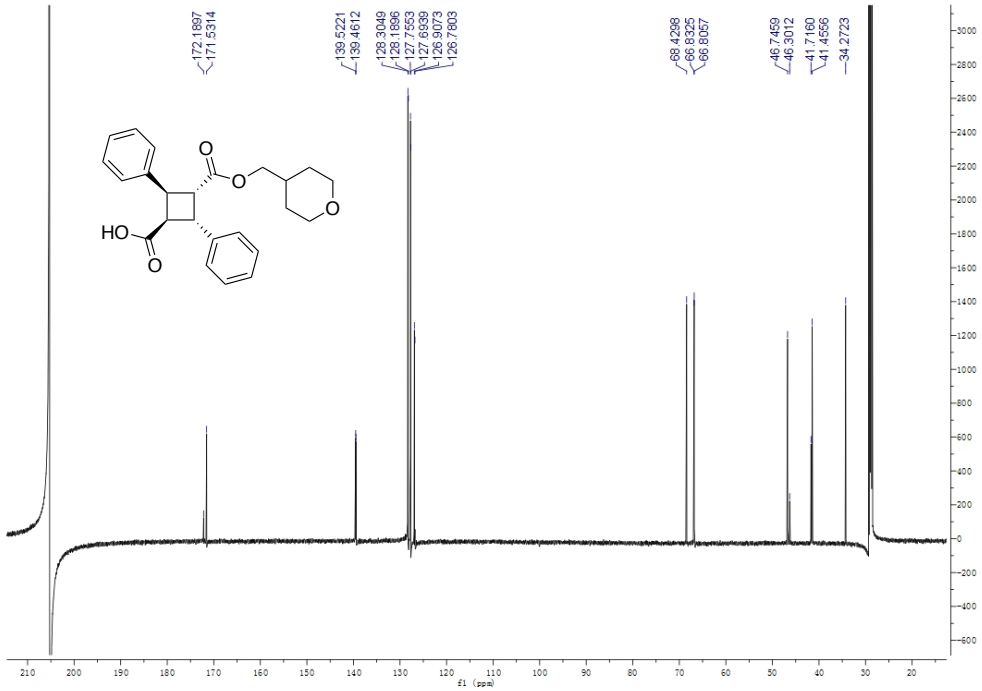
Di([1,1'-biphenyl]-3-yl) α-2,4-diphenylcyclobutane-1,3-dicarboxylate (4-3)



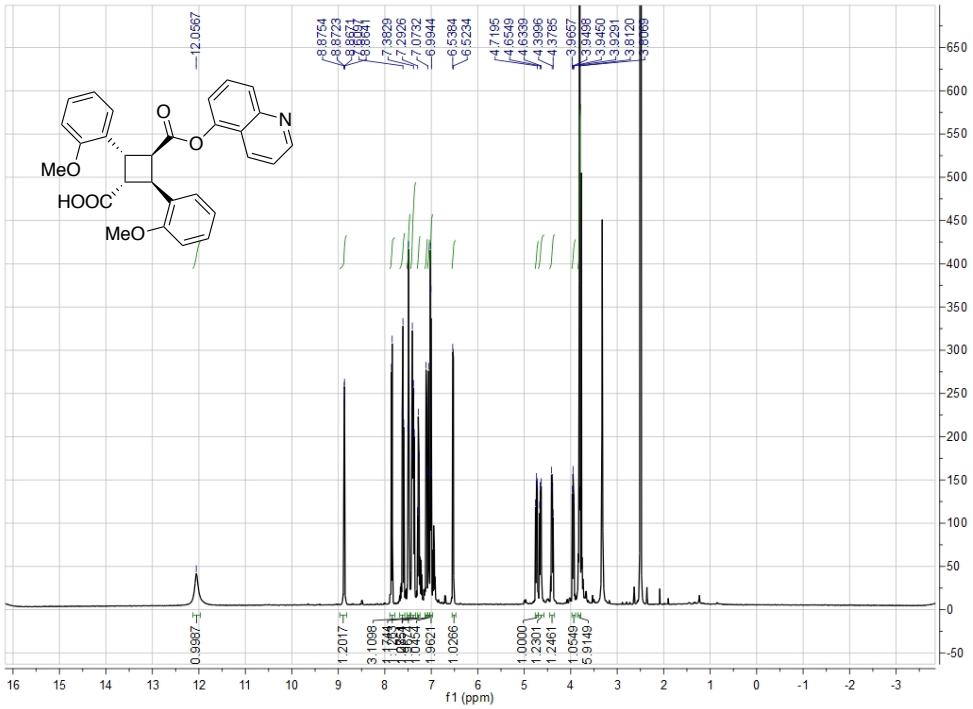


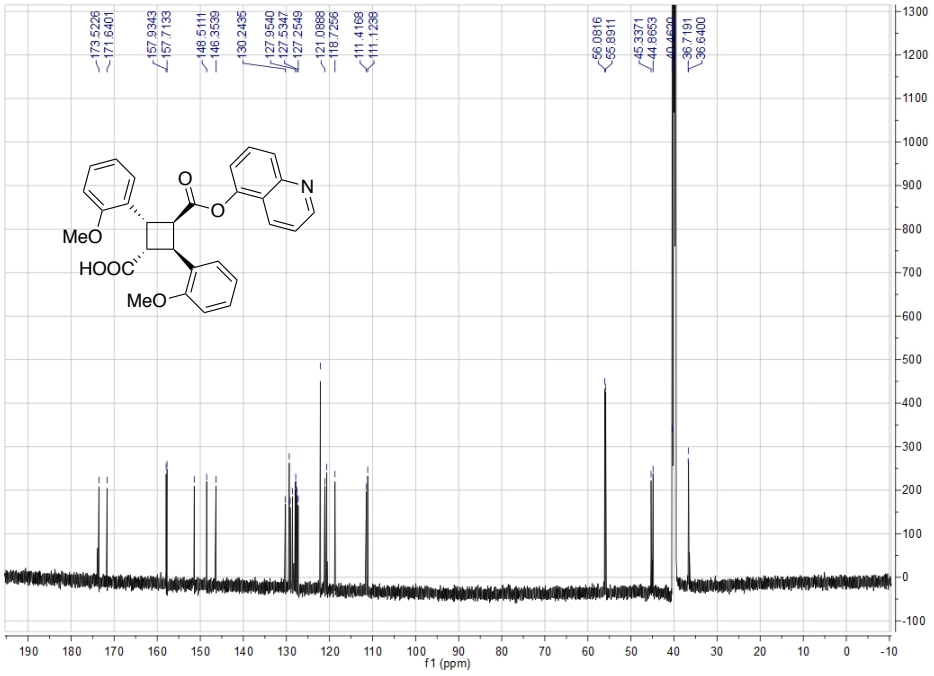
α-2,4-Diphenyl-3-(tetrahydro-2H-pyran-4-ylmethoxycarbonyl)cyclobutane-1-carboxylic acid (4-4)



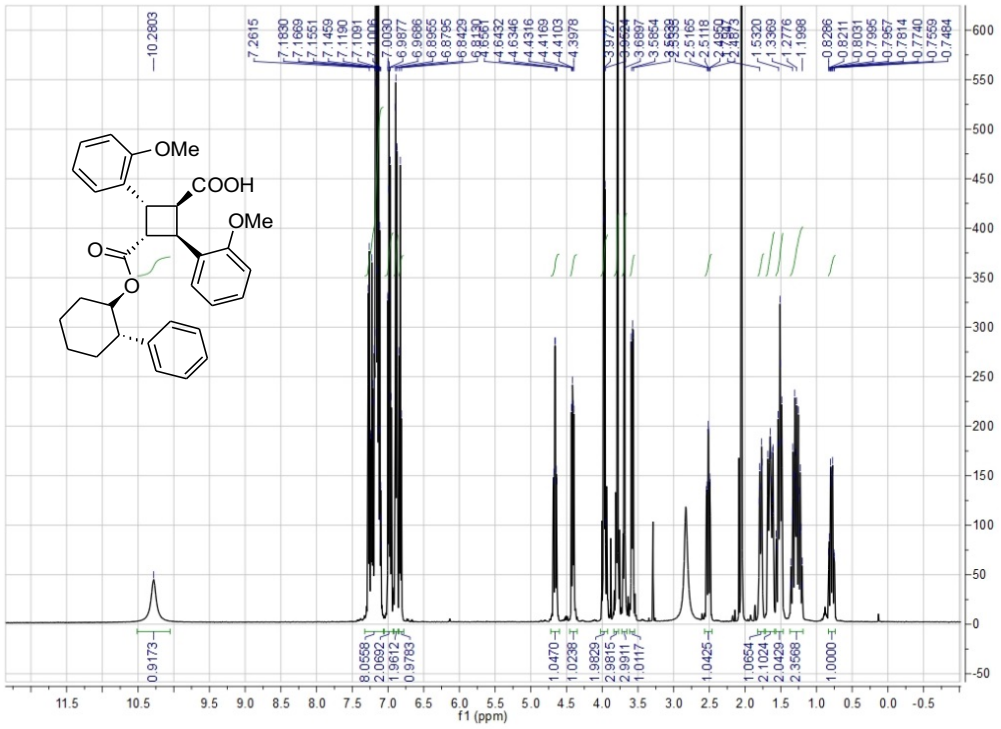


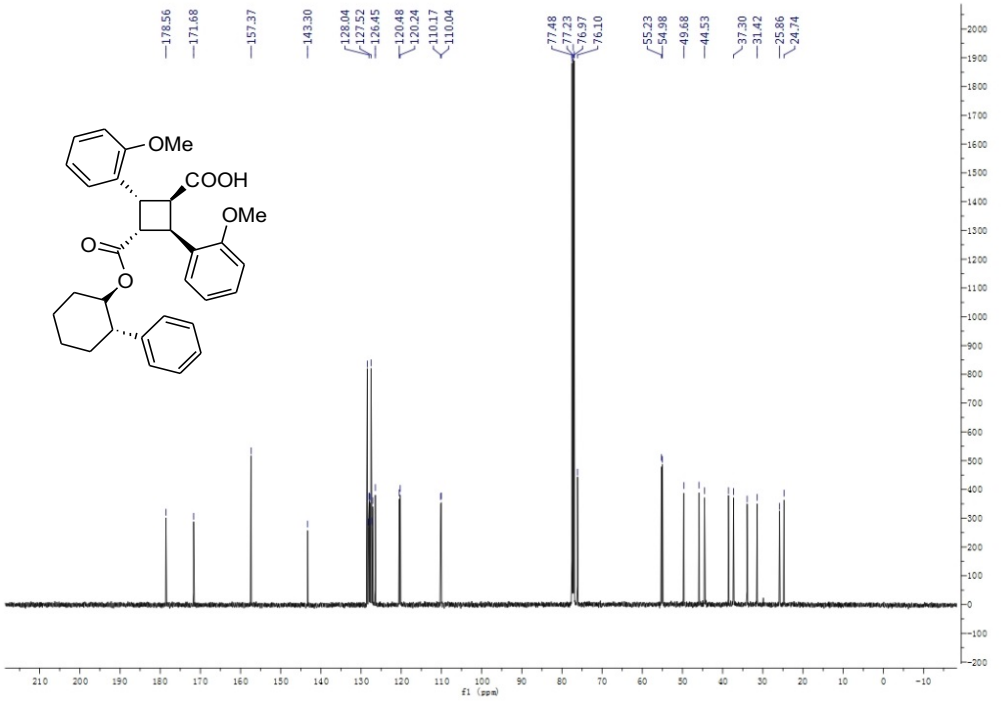
α-2,4-Bis(2-methoxyphenyl)-3-((quinolin-5-yloxy)carbonyl)cyclobutane-1-carboxylic acid (4-6)



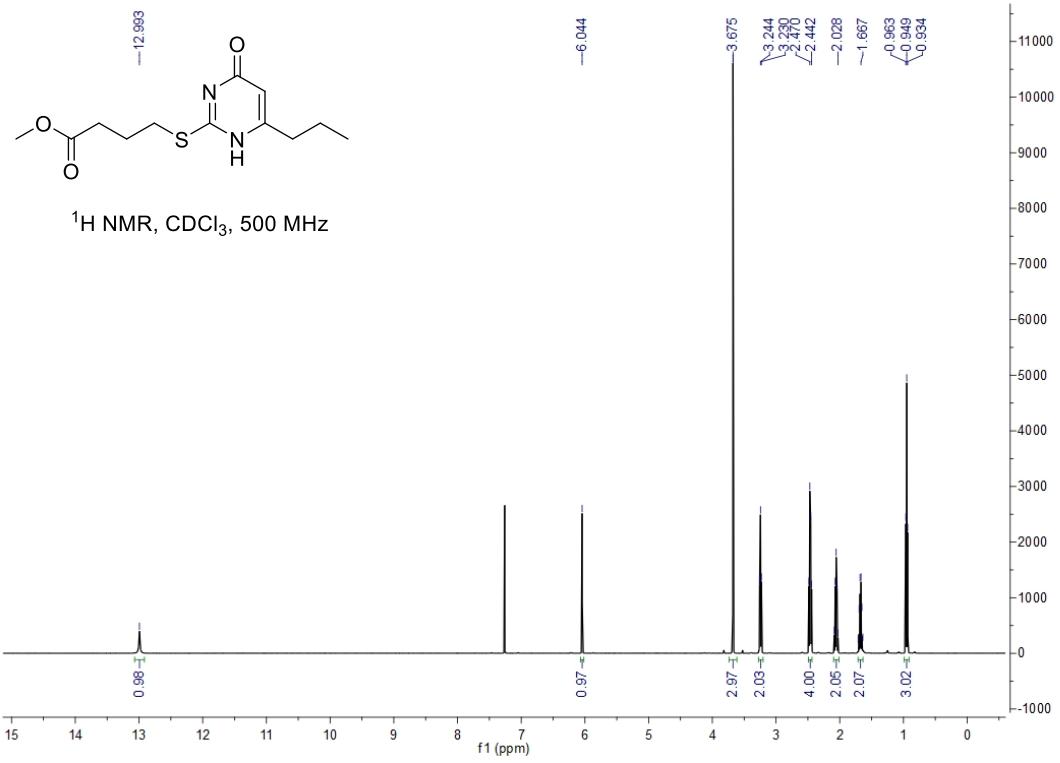


α-2,4-Bis(2-methoxyphenyl)-3-(2-phenylcyclohexyloxy)carbonyl)cyclobutane-1-carboxylic acid (4-7)

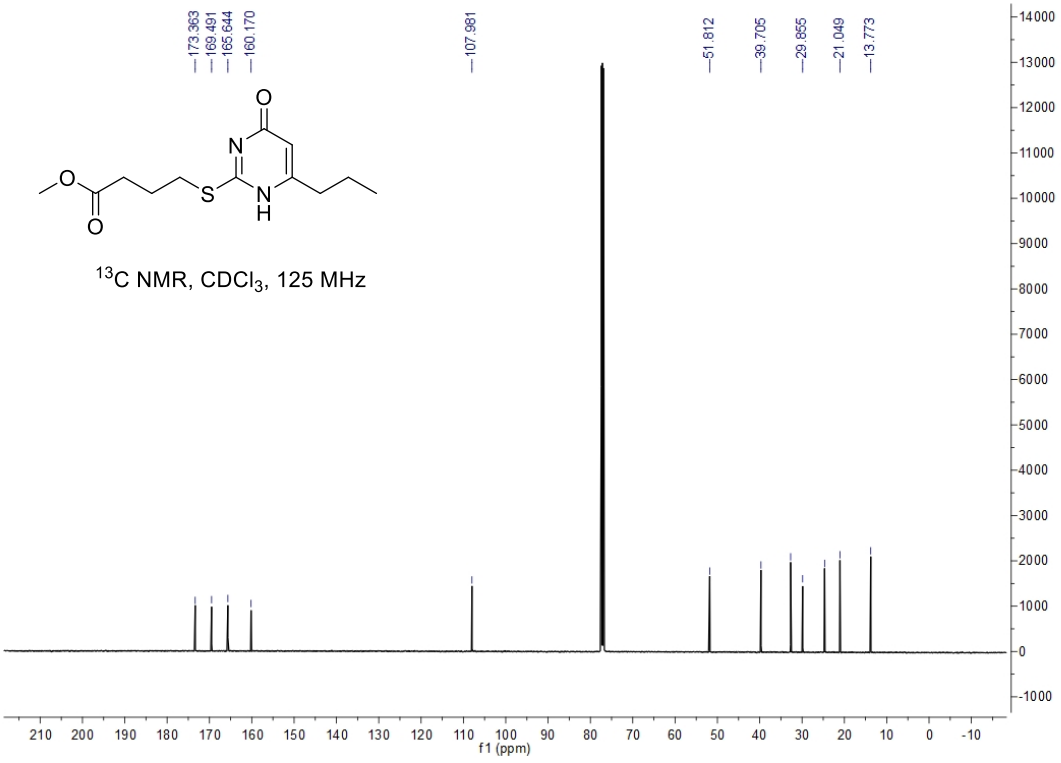




Methyl 4-(4-oxo-6-propyl-1,4-dihydropyrimidin-2-ylthio)butanoate (6-2)

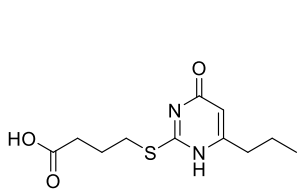


^{1}H NMR, $CDCl_3$, 500 MHz

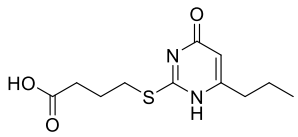


^{13}C NMR, $CDCl_3$, 125 MHz

4-(4-Oxo-6-propyl-1,4-dihydropyrimidin-2-ylthio)butanoic acid (6-3)

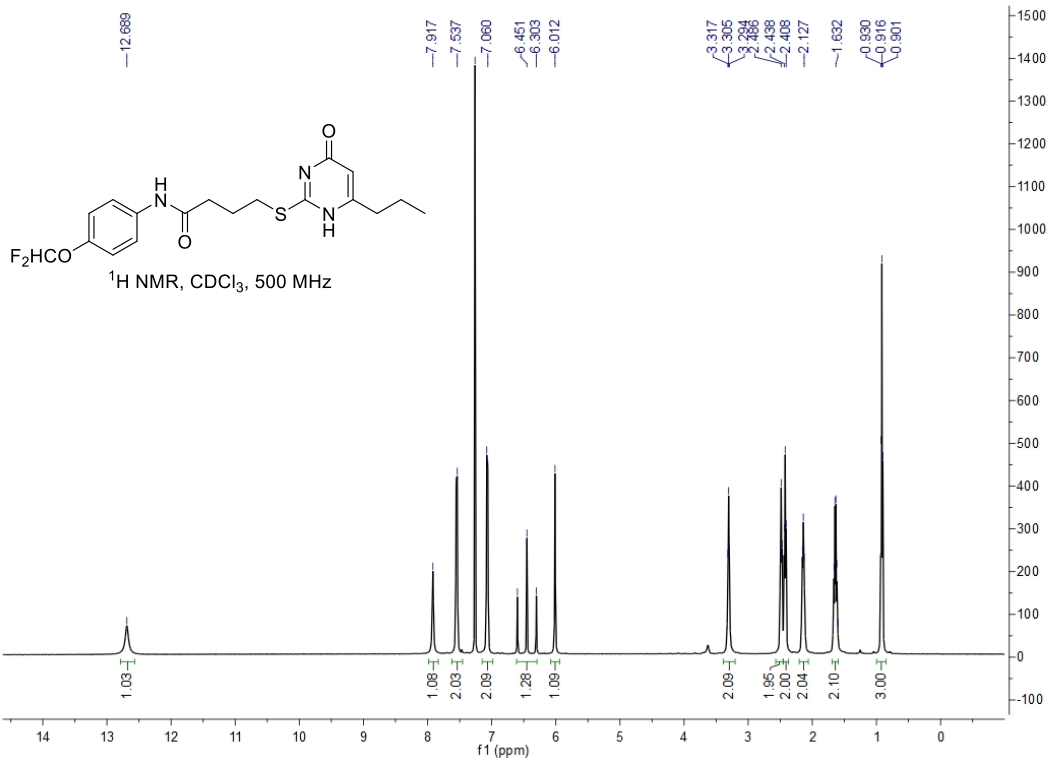


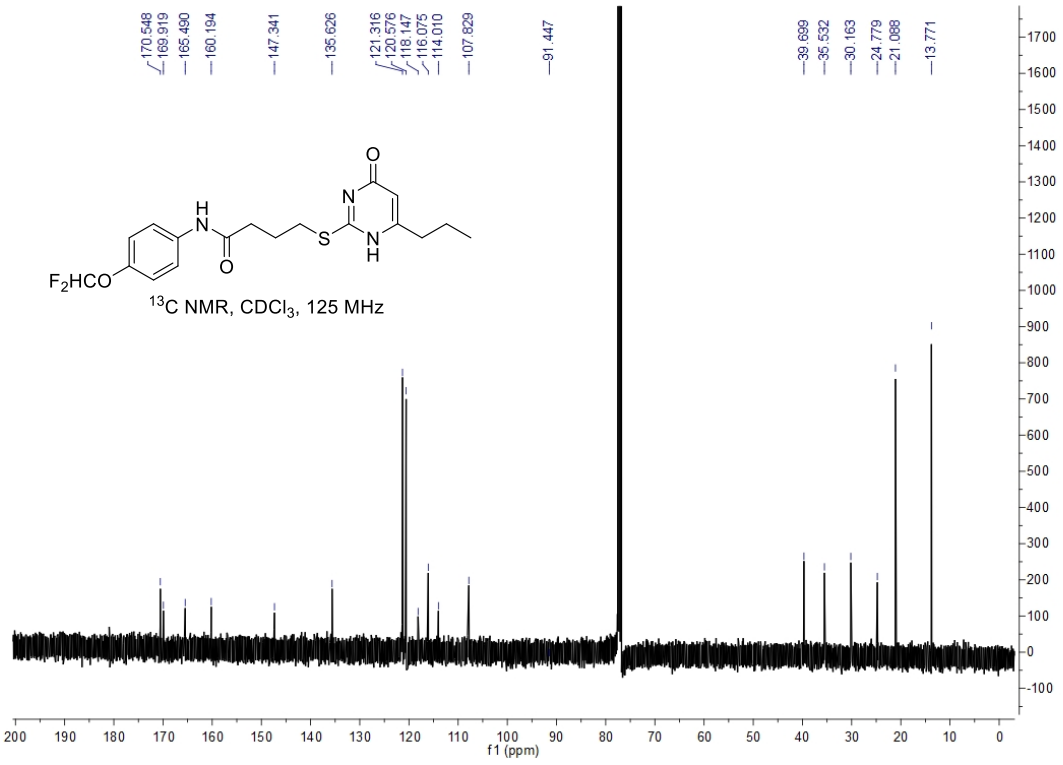
^{1}H NMR, $CDCl_3$, 300 MHz



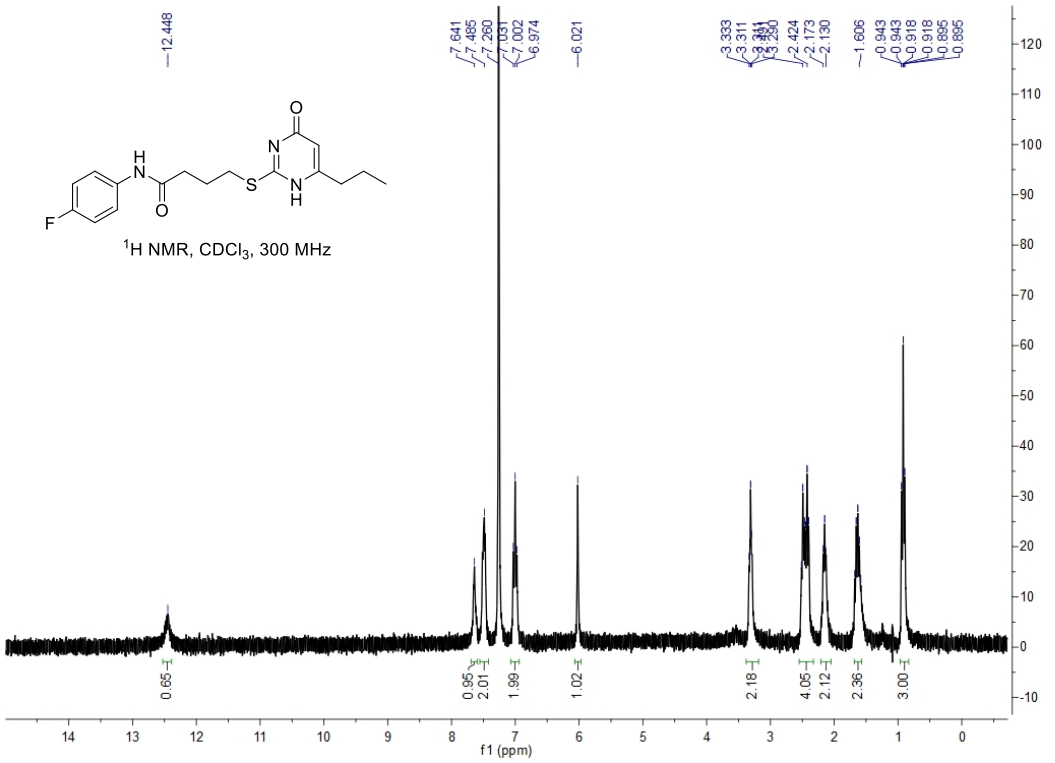
^{13}C NMR, $CDCl_3$, 175 MHz

_N_-(4-Difluoromethoxyphenyl)-4-(4-oxo-6-propyl-1,4-dihydropyrimidin-2-ylthio)butanamide (6-4)

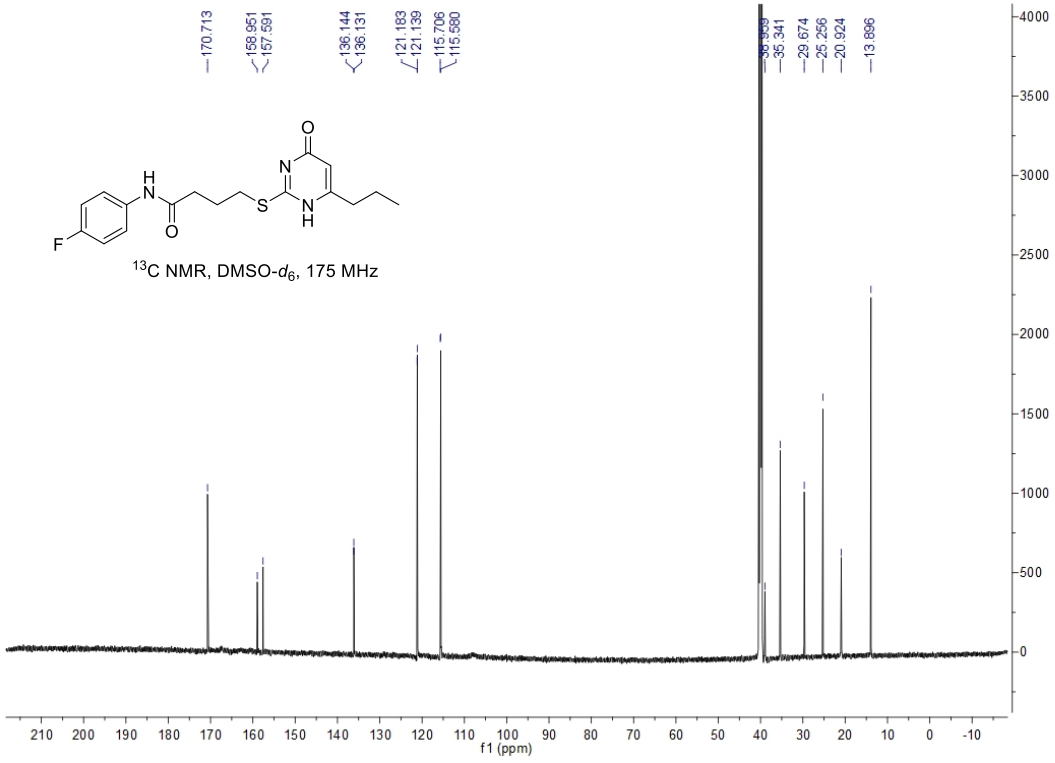




_N_-(4-Fluorophenyl)-4-(4-oxo-6-propyl-1,4-dihydropyrimidin-2-ylthio)butanamide (6-5)

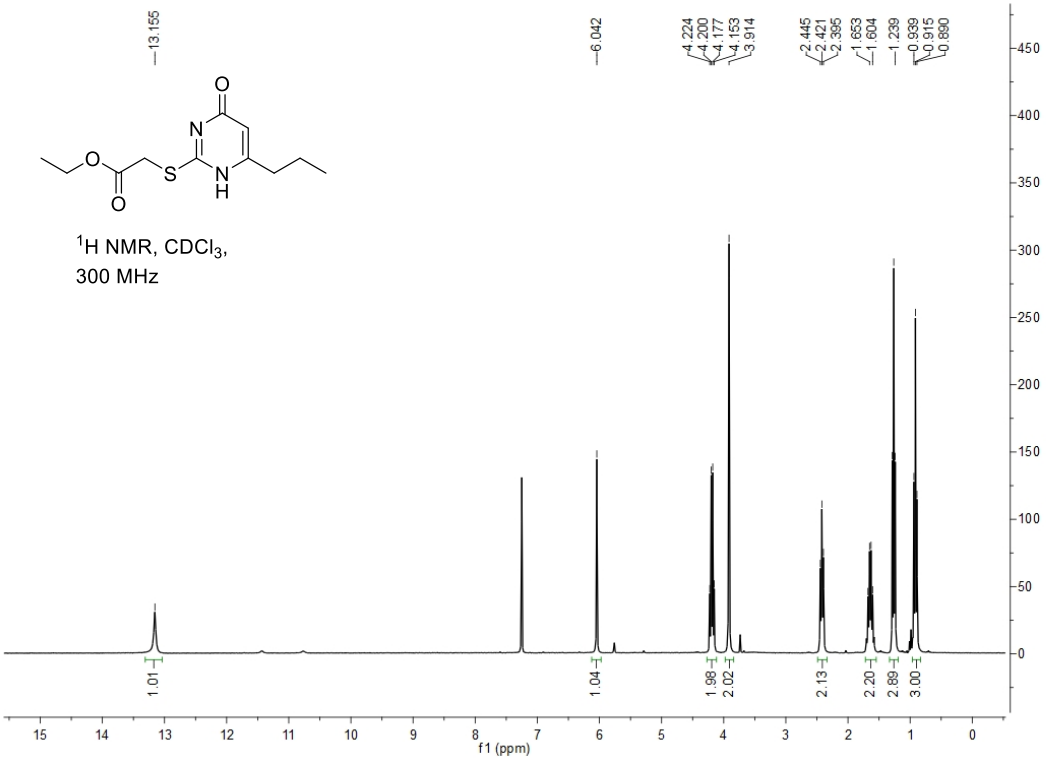


^{1}H NMR, $CDCl_3$, 300 MHz

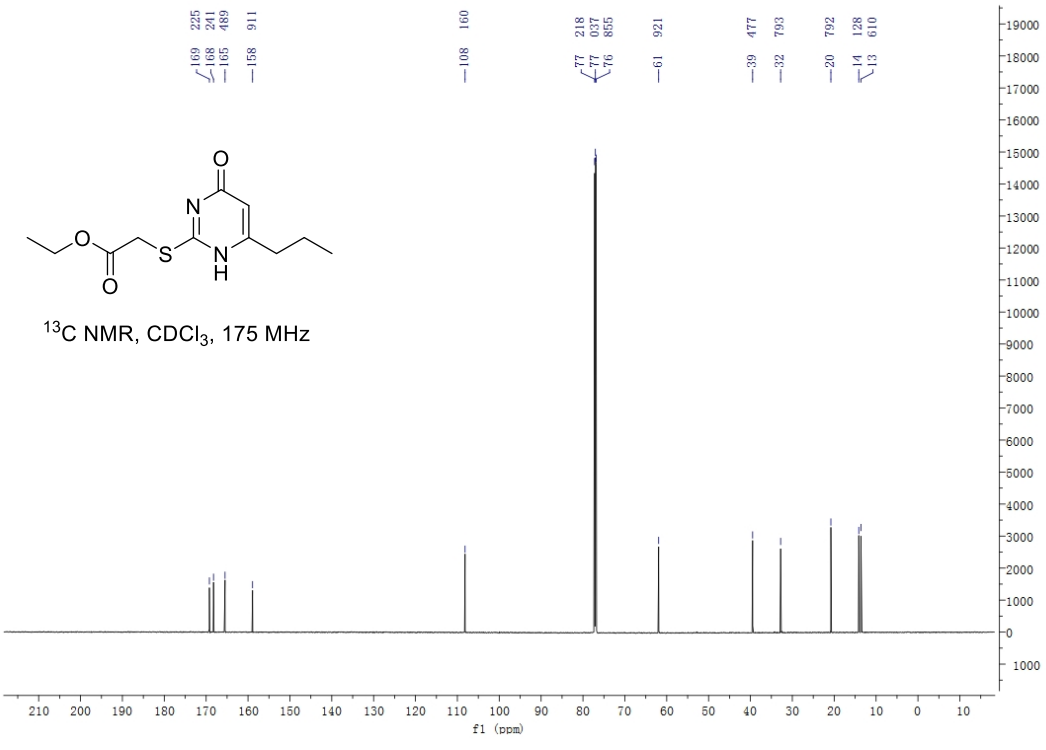


^{13}C NMR, DMSO-d_6, 175 MHz

Ethyl 2-(4-oxo-6-propyl-1,4-dihydropyrimidin-2-ylthio)acetate (7-2)

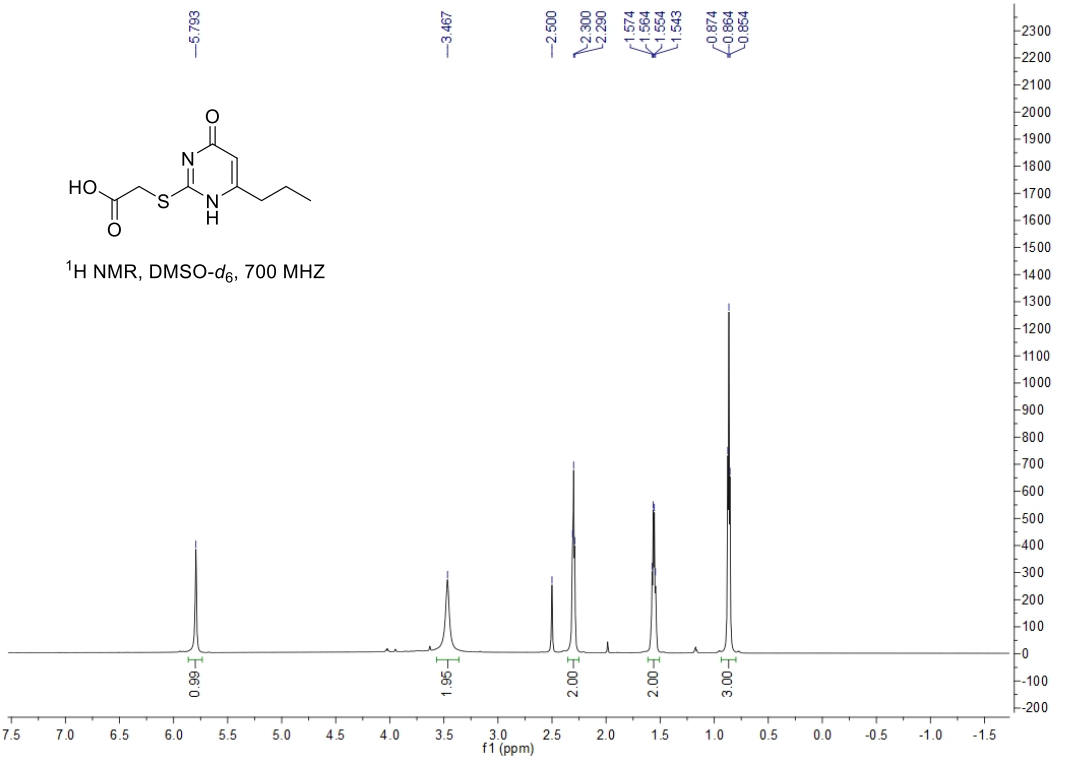


^{1}H NMR, $CDCl_3$, 300 MHz

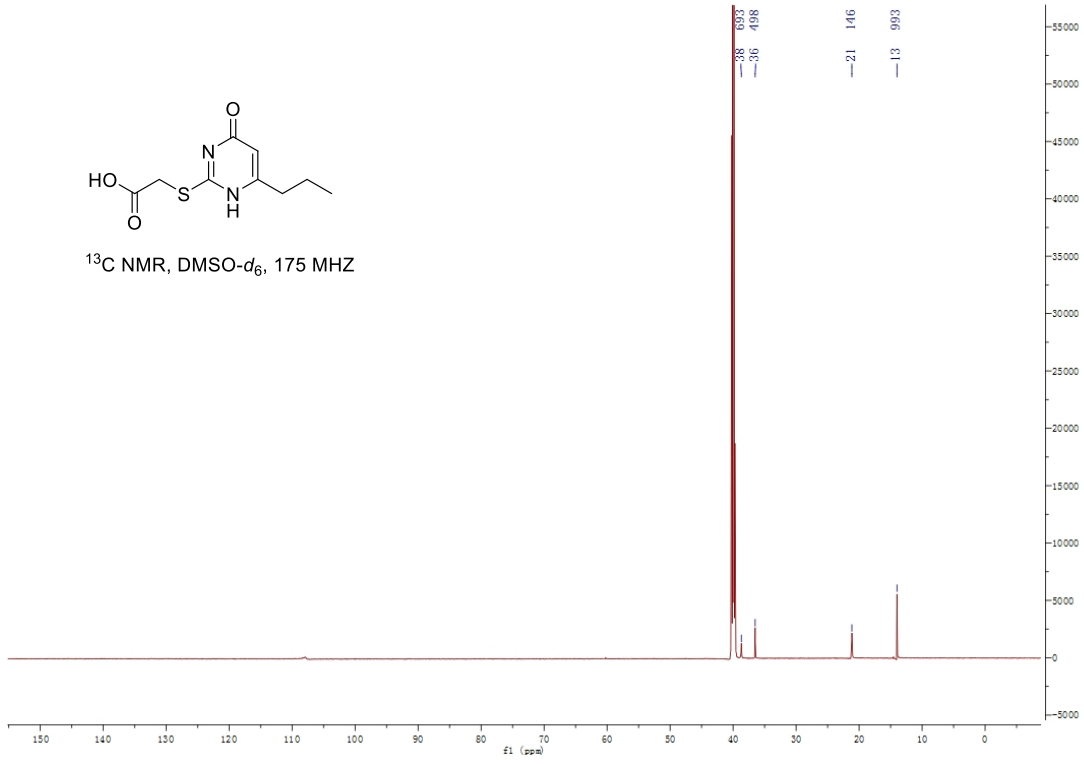


^{13}C NMR, $CDCl_3$, 175 MHz

2-((4-Oxo-6-propyl-1,4-dihydropyrimidin-2-yl)thio)acetic acid (7-3)

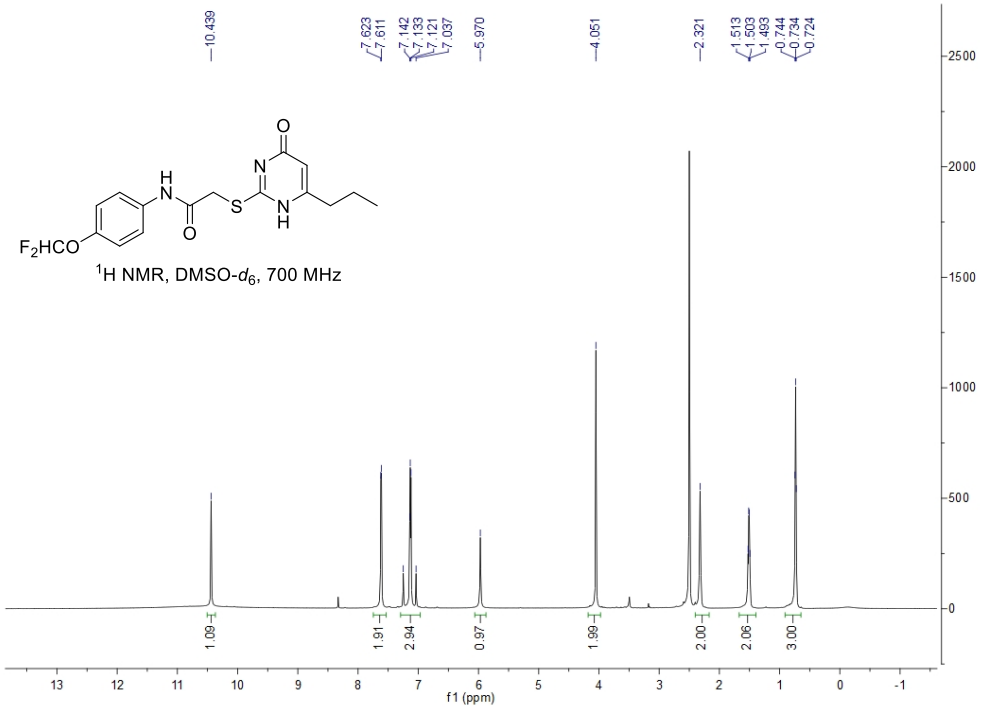


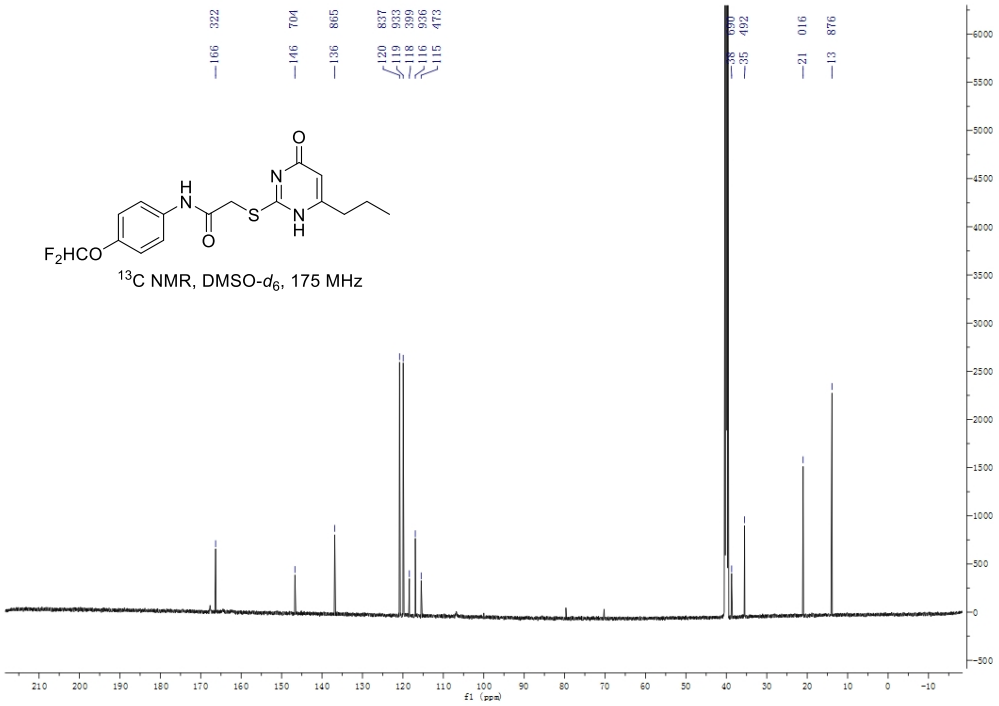
^{1}H NMR, DMSO-d_6, 700 MHZ



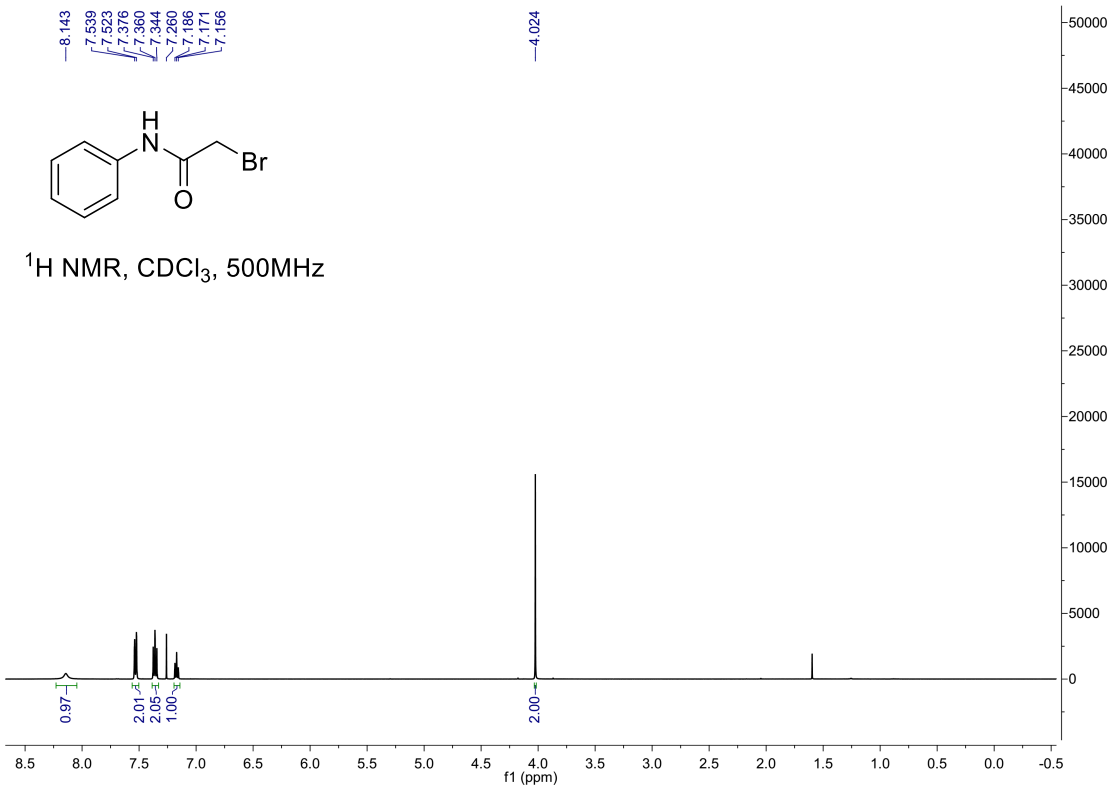
^{13}C NMR, DMSO-d_6, 175 MHZ

***N*-(4-Difluoromethoxyphenyl)-2-(4-oxo-6-propyl-1,4-dihydropyrimidin-2-ylthio)acetamide (7-4)**

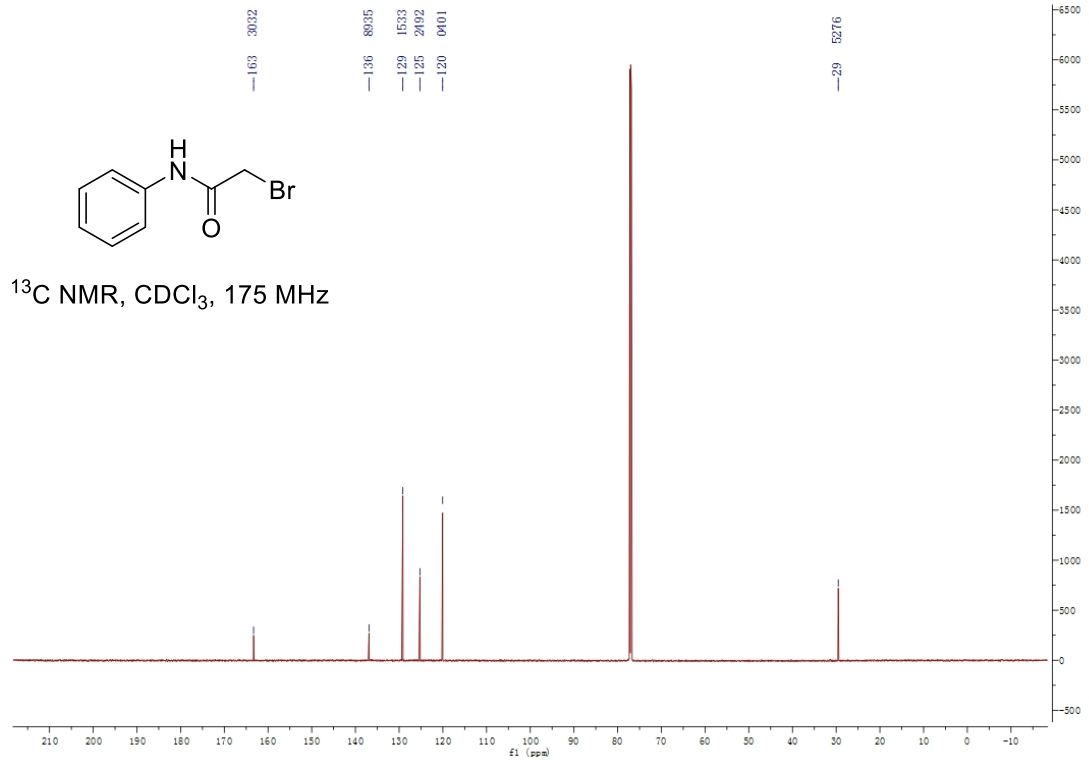




2-Bromo-*N*-phenylacetamide (8-3)

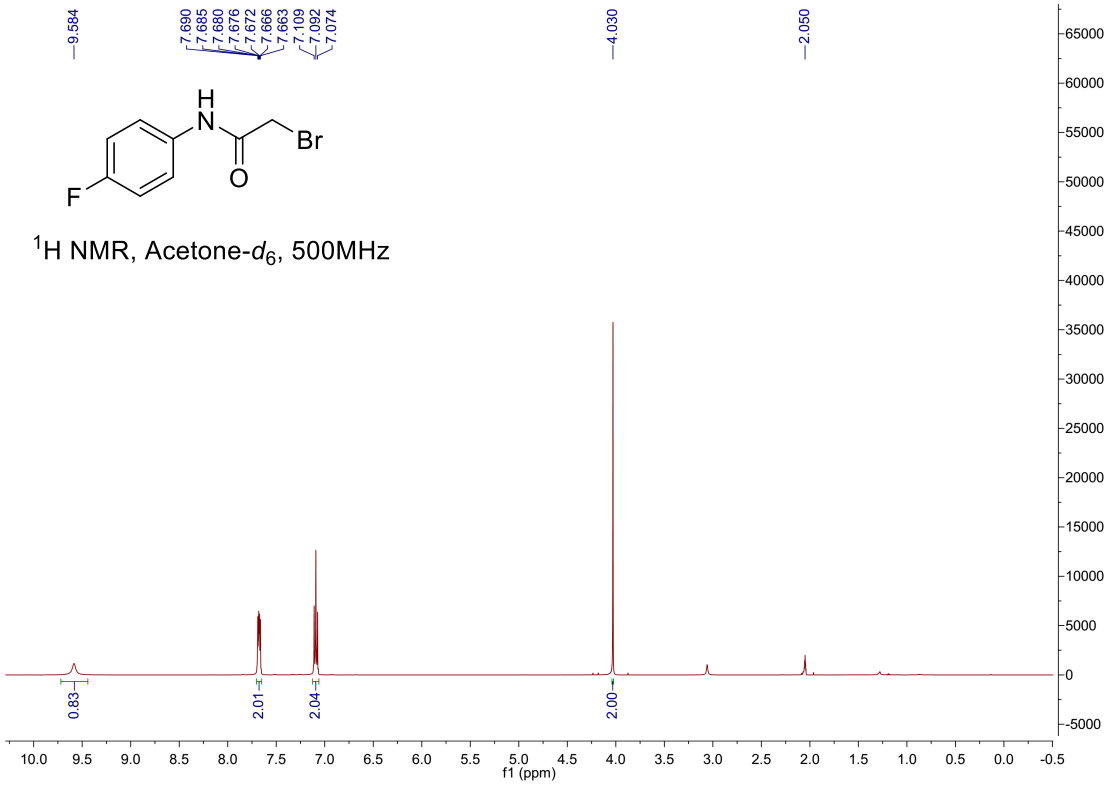


^{1}H NMR, $CDCl_3$, 500MHz

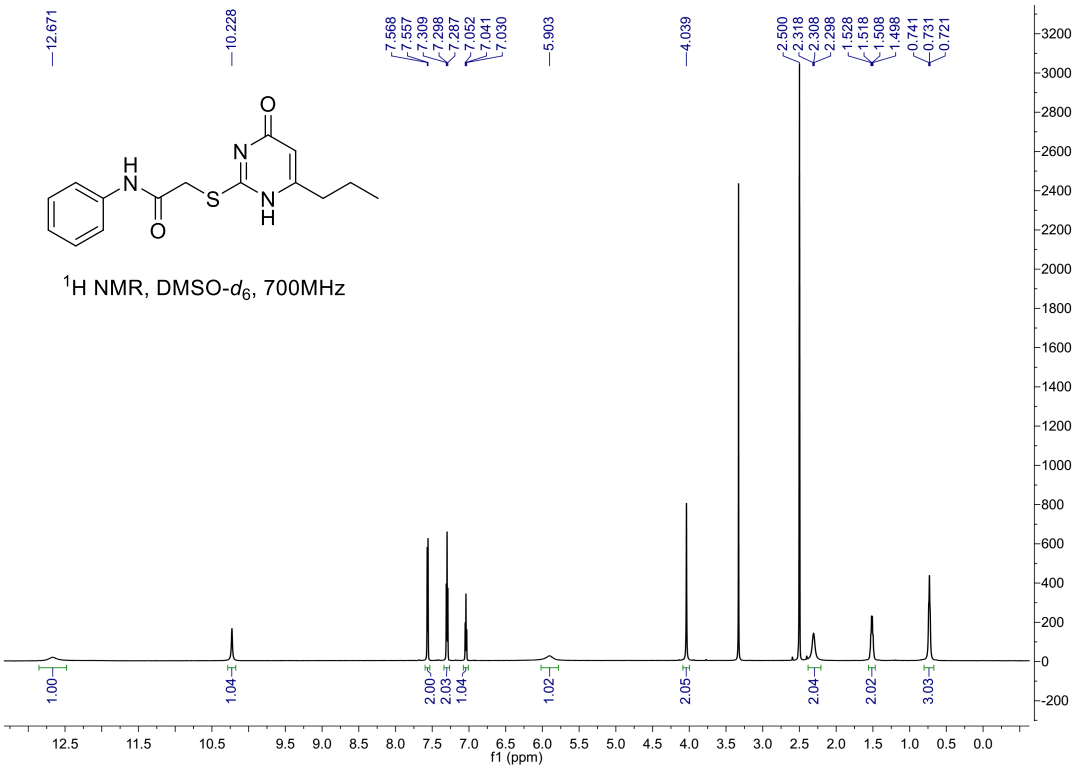


^{13}C NMR, $CDCl_3$, 175 MHz

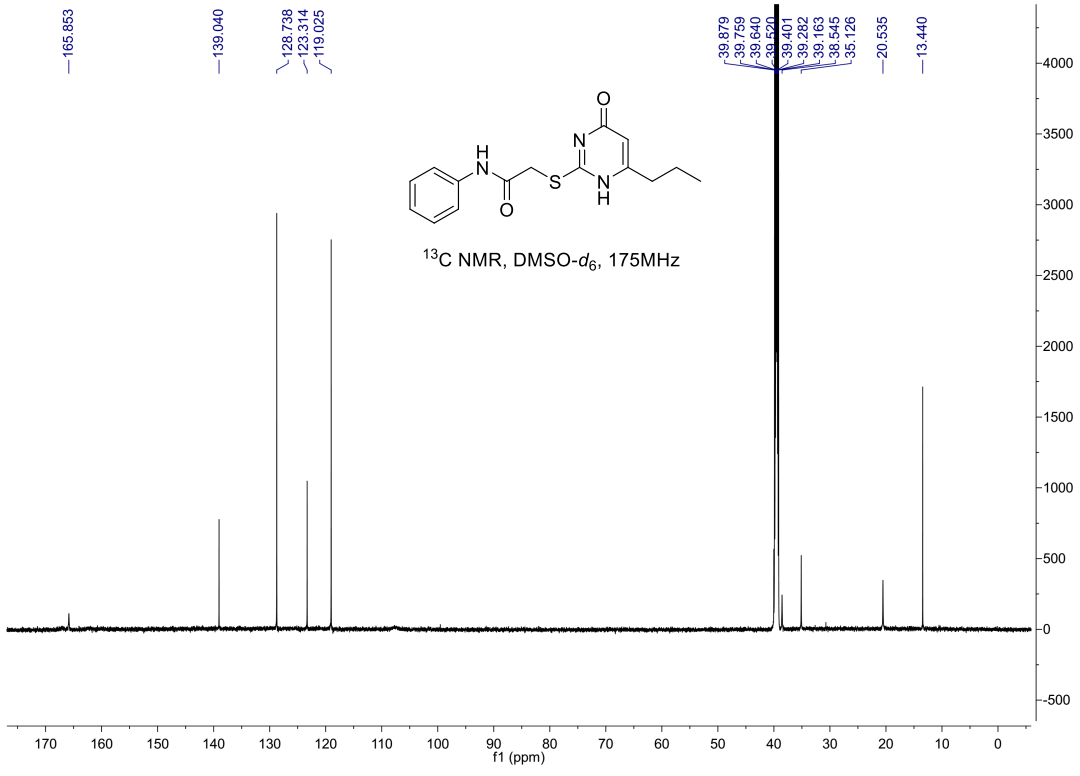
2-Bromo-*N*-(4-fluorophenyl)acetamide (8-4)



2-(4-Oxo-6-propyl-1,4-dihydropyrimidin-2-ylthio)-*N*-phenylacetamide (8-5)



^{1}H NMR, DMSO-d_6, 700MHz



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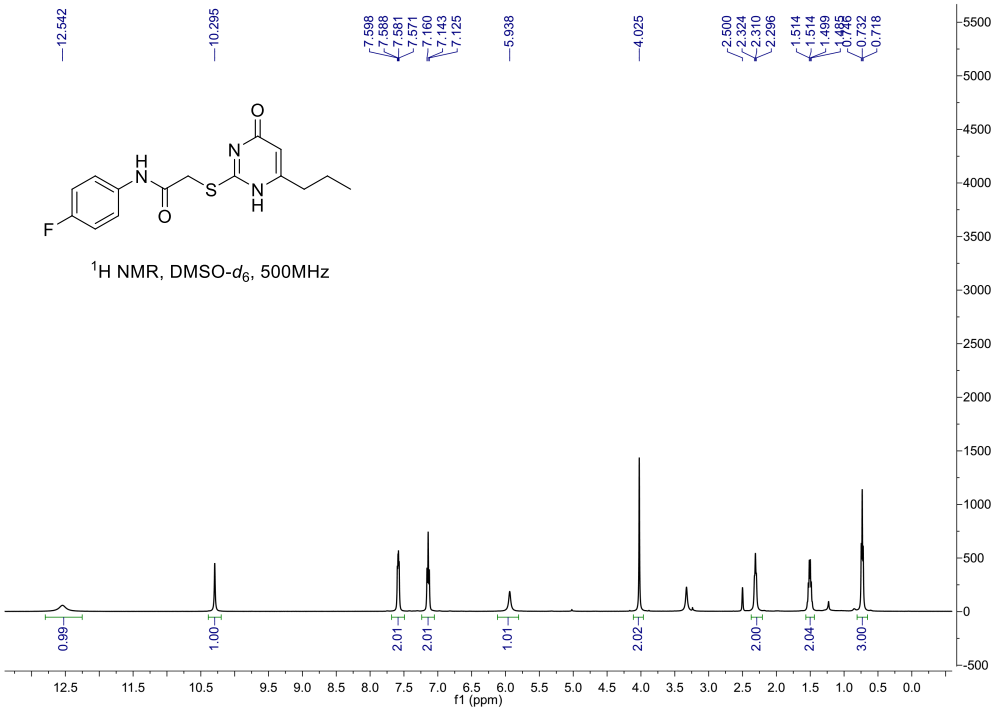
N-(4-Fluorophenyl)-2-(4-oxo-6-propyl-1,4-dihydropyrimidin-2-ylthio)acetamide (8-6)



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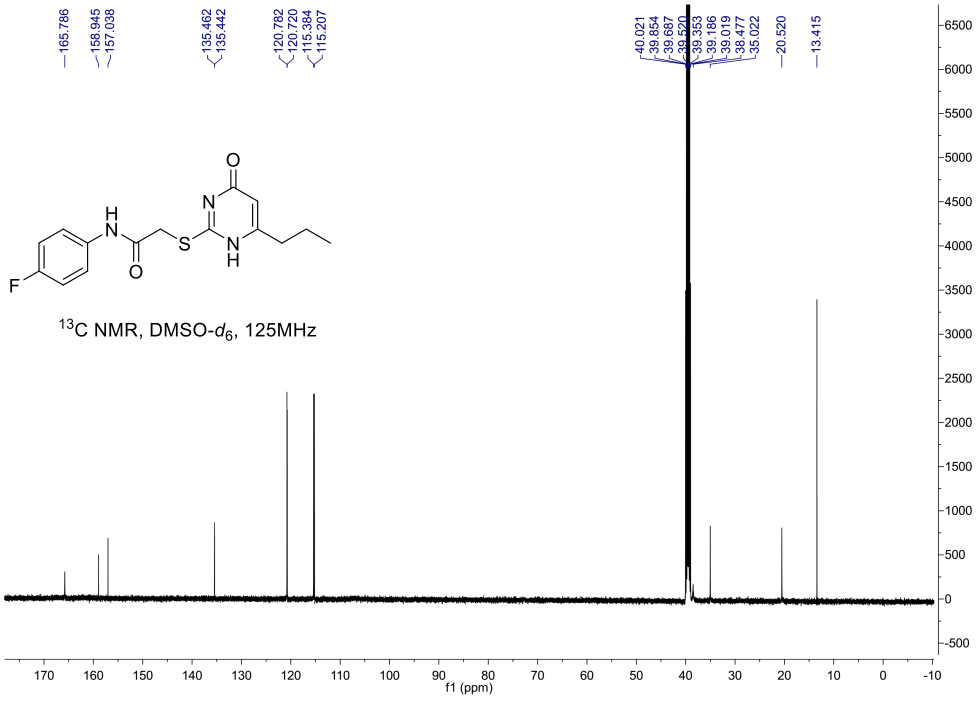

^{13}C NMR, DMSO-d_6, 125MHz